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13. ABSTRACT ***(Maximum 200 Words)***

Under this contract, pharmacokinetic, pharmacodynamic, bioavailability, and metabolism studies were conducted with the anti-malarial agent, artelinic acid. During these investigations, artesunic acid and arteether served as positive control agents. Major findings indicated that the oral bioavailability of artelinic acid in dogs was 67%; artelinic acid was extensively metabolized by dogs following either iv or oral administration; radioactivity derived from [^{14}C]artelinic acid underwent biliary excretion; a "no effect" dose of artelinic acid for the production of neurotoxicity in rats was 20 mg/kg/day, qd x 14; a "no effect" dose of artelinic acid for the production of neurotoxicity in dogs was < 1mg/kg/day, when given orally for 14 consecutive days; an injectable formulation of artelinic acid/lysine salt was well tolerated by dogs given daily iv doses for 7 consecutive days; the maximum tolerated dose (MTD) of artelinic acid for rats given a single iv dose was ≥ 80 and < 160 mg/kg; the MTD of artesunic acid for rats given a single iv dose was ≥ 200 and < 400 mg/kg; no neurohistopathological lesions were observed for rats given ≤ 37.5 mg/kg/day, qd x 7, of artelinic acid or up to 150 mg/kg/day, qd x 7, of artesunic acid.

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1.0 INTRODUCTION

The work scope of this contract involved the performance of studies in rats and dogs on the pharmacokinetic and pharmacodynamic properties of drugs under clinical development by the U.S. Army Medical Research and Development Command. The pharmacokinetic aspect of these studies involved an investigation of the absorption, disposition, metabolism (biotransformation), and elimination of test compounds in experimental animals. The pharmacodynamic aspect involved relating certain measured parameters, for example, the production of methemoglobin, to blood and plasma levels of the test compound and/or its metabolites, or assessing various toxicological parameters such as clinical signs, clinical pathological changes, or histopathological changes, occurring after administration of a test compound. The information derived from these studies was intended to provide a data base for establishing an appropriate species and appropriate doses for subsequent subchronic and chronic toxicity studies, to predict for possible organ toxicities which might occur, and to generate data required by the Food and Drug Administration prior to submission of a Notice of Claimed Investigational Exemption for a New Drug (IND) and New Drug Applications, Human Use (NDA).

During the term of the contract, several investigations were conducted with the anti-malarial agent, artelinic acid. One study also involved a related anti-malarial agent, artesunic acid. The investigations conducted under the contract were as follows:

- Task Order SR98-2: Bioavailability, Pharmacokinetics, and Identification of Metabolites of Artelinic Acid in Dogs
- Task Order SR98-3: Effect of Artelinic Acid on Rats after Oral Administration for 14 Days
- Task Order SR99-1: Effect of Artelinic Acid on Dogs after Oral Administration for 14 Days
- Task Order SR00-1: Study of Injectable Artelinic Acid in Dogs
- Task Order SR01-1: Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

A list of personnel who were involved in these investigations and who received pay for their research efforts is included in Appendix A.

2.0 RESEARCH ACCOMPLISHMENTS FOR EACH TASK ORDER

2.1 Task Order SR98-2: Bioavailability, Pharmacokinetics, and Identification of Metabolites of Artelinic Acid in Dogs

2.1.1 Objective

Early in this century, with the discovery of quinine, malaria appeared to be one of the few diseases for which a specific cure existed. As a result, however, of the development of insecticide resistance by the Anopheles mosquito and the development by this parasite of resistance to chloroquine and mefloquine, malaria remains a major health problem in many areas of the world.[1] Qinghaosu (QHS), also known as artemisinin, was initially isolated and characterized by the Chinese and found to be an effective antimalarial agent.[2] This compound is a sesquiterpene lactone with an endo-peroxide bridge. The ethyl ether (arteether) of reduced QHS, dihydroqinghaosu (DQHS), and the succinate hemiester of DQHS, artesunic acid, are also effective in the treatment of severe cases of multi-drug resistant malaria.[3] These compounds, while therapeutically active, have several limitations, one of which is their thermal lability.

In the search for better antimalarial agents, artelinic acid, a semisynthetic water-soluble derivative of QHS, was developed. The sodium salt of artelinic acid, sodium artelinate, has been reported to be effective against chloroquine-resistant *P. falciparum* and to be superior to QHS against *P. berghei*.[4] Sodium artelinate has also been reported to be 500- to 1000-fold more stable than α-artesunic acid when maintained at comparable temperature and (aqueous buffer) pH conditions.[5]

The purposes of this study were to determine the absorption, bioavailability, elimination, and pharmacokinetics of artelinic acid, and to identify the metabolites of the compound, following single dose intravenous or oral administration of [^{14}C]artelinic acid to male and female dogs. In addition, the possible cardiotoxicity of artelinic acid was assessed using electrocardiography.

2.1.2 Materials and Methods

2.1.2.1 Test Article. The test article used in this study was artelinic acid [4-(10'-dihydroartemisininoxymethyl)benzoic acid hemihydrate]. The structure of artelinic acid is shown in Figure 1; the position of the [^{14}C] on the radiolabeled compound is indicated.

Unlabeled artelinic acid (Bottle No. BP11387; MFG Code No. NJ33-55-1) was synthesized by Starks Associates, Inc. (Buffalo, NY) and supplied by Walter Reed Army Institute of Research (WRAIR; Washington, DC). [16-^{14}C]artelinic acid (Lot No. CT-9297-67) was synthesized by Research Triangle Institute (Research Triangle Park, NC). The stated specific activity and radiochemical purity of the [^{14}C]artelinic acid were 26.2 μCi/mg and 97%, respectively; the radiochemical purity of the compound was verified at Southern Research upon

receipt. Unlabeled artelinic acid was stored at approximately -20°C; [^{14}C]artelinic acid was stored in an ultra-cold freezer (-70°C).

2.1.2.2 Test Article Formulation. The dose formulation of [^{14}C]artelinic acid for iv administration was prepared to contain approximately 10 mg of artelinic acid per mL and to have a radioactivity content of approximately 20 μCi/mL. For preparation, unlabeled artelinic acid (461.80 mg) and [^{14}C]artelinic acid (38.24 mg) were dissolved in 2.6 mL of 0.5% sodium carbonate in sterile water. Saline (0.9%) was then added to a volume of 45 mL. The pH of the solution was adjusted to approximately 9.0 with 0.1 M hydrochloric acid and additional saline was added to a final volume of 50 mL. The dose formulation was subsequently filtered, using 0.45 □m nylon filters, 25 mm (Gelman Sciences; Ann Arbor, MI), and stored at room temperature prior to use.

The dose formulation of [^{14}C]artelinic acid for oral (po) administration was prepared to contain approximately 5 mg of artelinic acid per mL and to have a radioactivity content of approximately 10 μCi/mL. For preparation, unlabeled artelinic acid (461.80 mg) and [^{14}C]artelinic acid (38.20 mg) were homogenized in 35 mL of 1% carboxymethylcellulose: 0.5% Tween 80 using a Potter-Elvehjem homogenizer. The suspension was then sonicated for approximately 30 seconds and additional 1% carboxymethylcellulose: 0.5% Tween 80 was added to a final volume of 100 mL. The formulation was mixed well by inversion and stored refrigerated prior to use. On the day of use, the dose formulation was removed from the refrigerator and stirred at room temperature until it appeared, by visual inspection, to be homogenous.

The dose formulations of [^{14}C]artelinic acid for iv or po administration were prepared 3 days prior to use.

2.1.2.3 Dose Formulation Analysis. Upon preparation, portions of each [^{14}C]artelinic acid dose formulation were analyzed for chemical concentration, radiochemical purity, and radioactivity content. Prior to analysis, appropriate dilutions of the iv and po formulations were made. For the chemical concentration and radioactivity content analyses, the iv dose formulation was diluted 1:100 in 0.9% sodium chloride; dilutions (1:100) of the po formulation were made in water and then 65% methanol: 35% 0.1M ammonium acetate. For the radiochemical purity determinations, the iv dose formulation was diluted 1:50 in saline; the po formulation was diluted 1:50 in the HPLC mobile phase (65% methanol:35% 0.1M ammonium acetate). To verify the concentration of artelinic acid in each dose formulation, dilutions (1:100) of the samples which were removed from the top, middle, and bottom of each formulation were analyzed by HPLC using a previously validated method. The conditions of analysis were as follows:

Column:	Bondapak C_{18}, 10 μm, 300 mm x 3.9 mm (Waters Corporation, Milford, MA)
Mobile Phase:	65% Methanol:35% 0.1M ammonium acetate
Elution:	Isocratic
Temperature:	Ambient
Flow rate:	1.5 mL/min
Detection:	UV absorbance at 235 nm

The concentration of artelinic acid in each dose formulation sample was calculated from the response (peak area) of standard solutions of artelinic acid, prepared in sodium carbonate/0.9% saline for the iv formulation analysis or methanol for the po formulation analysis, using linear regression analysis.

To determine radiochemical purity, a portion (200 µL) of a 1:50 dilution of each dose formulation was assayed by HPLC under the conditions described for the chemical concentration verification with the addition that eluting sample components were detected using an in-line radio-chromatography detector.

The radioactivity content of each dose formulation was determined following combustion and radioanalysis of quadruplicate aliquots (0.1 mL) of 1:100 dilutions of top, middle, and bottom samples obtained from each formulation.

2.1.2.4 Test Animals. The eight purebred beagle dogs (four male and four female) designated for use in this study were received from Covance Research Products, Inc. (Cumberland, VA). The dogs were 6-11 months of age when they arrived at Southern Research Institute (Southern Research) on December 30, 1998. Individual animal identification was by ear tattoo. The beagle dog is an accepted species to support clinical studies of drugs intended for use in humans. On the day of dosing (1/18/99), the dogs were approximately 7-12 months old.

Upon arrival, the dogs were placed in quarantine, and each dog was given a physical examination. The physical included a thorough examination of all external surfaces, organs, and orifices, a check for internal parasites (by fecal examination) and external parasites, blood collection for determination of selected clinical pathology parameters, and measurement of body weight. There were no significant findings indicative of poor health. Each dog was allowed access to Certified Canine Diet #5007 (PMI Feeds, Inc.; St. Louis, MO) for approximately 2 hours each day during the quarantine and study periods. Tap water (Birmingham public water supply) was available *ad libitum* to each animal during the quarantine and study periods. No known contaminants were present in the food or water that would affect the study outcome. The dogs were housed individually in stainless steel cages in animal rooms that were maintained between 65-72 °F with a relative humidity of 33-61% during the study period; temperature and humidity readings were recorded twice a day during the study period. Room lights were controlled by an automatic timer set to provide 12 hours of light (0600 to 1800 hours, CST) and 12 hours of dark per day. Cage size and animal care conformed to the Standard Operating Procedures of Southern Research, *The Guide for the Care and Use of Laboratory Animals*, 7th edition[6] and Public Law 99-198.

2.1.2.5 Dosing. On the day prior to dosing, dogs were given their daily ration of food in the afternoon and then fasted overnight. Dogs (two male and two female) in Group A were administered a single iv dose of approximately 10 mg/kg of [^{14}C]artelinic acid by slow-push injection over 2-3 minutes into a peripheral vein; the dose volume was 1 mL/kg. Dogs (two male and two female) in Group B were administered a single po dose of approximately 10 mg/kg of [^{14}C]artelinic acid by oral gavage in a dose volume of 2 mL/kg. All dosing was accomplished between 9:00 a.m. and 10:00 a.m., CST.

2.1.2.6 Sample Collection. Blood samples were collected from each dog in Group A (iv dose) at approximately 5, 15, and 30 minutes; 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 192 hours after dosing; blood samples were collected from each dog in Group B (po dose) at approximately 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 192 hours after dosing. The volume of blood collected at each time point was approximately 4 mL for samples obtained through the first 72 hours after dosing and approximately 10 mL for samples collected beyond 72 hours after dosing.

All blood samples were collected from the jugular vein of unanesthetized animals into tubes containing heparin and placed on ice upon collection. Approximately 1.5 mL of each blood sample collected during the first 72 hours after dosing was removed; aliquots of each sample were radioassayed, and approximately 1 mL of each sample was sent to the Sponsor. For blood samples collected beyond 72 hours after dosing, approximately 7-8 mL of each sample was removed; a small portion of each sample was radioassayed, and 1 mL of each sample was sent to the Sponsor. The remainder of each blood sample was saved for metabolite identification or centrifuged to obtain plasma. Blood and plasma samples were stored at approximately -80°C.

Urine and feces were scheduled to be collected from all dogs at approximately 0-12, 12-24, 24-48, 48-72, 72-96, 96-120, 120-144, 144-168, and 168-192 hours. At each excreta collection, the cage pan was rinsed with water, and the rinse was saved. Urine samples were stored at approximately -20°C prior to radioanalysis and at approximately -80°C after radioanalysis. The cage rinse samples were stored refrigerated prior to radioanalysis and at approximately -80°C after radioanalysis. Feces samples were stored at -80°C prior to processing for radioanalysis; fecal homogenates were stored at -80°C.

2.1.2.7 Measurements. Dogs were observed for adverse clinical signs on the day of dosing (observations were recorded at 3-4 time points for each dog) and at least twice daily thereafter for the remainder of the study.

Electrocardiogram readings were recorded for each dog prior to dosing and at approximately 2 and 48 hours postdosing using a Burdick Eclipse 800 electrocardiograph. Standard leads (I, II, III, $_a$VR, $_a$VF, $_a$VL) were obtained by placing electrodes on all four limbs of each animal. The EKG tracings were evaluated by a clinical veterinarian. Reported parameters were derived from manual analysis of the individual EKG tracings.

2.1.2.8 Sample Analysis. Duplicate portions (0.1 mL) of whole blood were placed in oxidizer cones, combusted, and the resultant samples were radioassayed.

Duplicate portions (0.1 mL) of plasma were placed in oxidizer cones, combusted, and the resultant samples were radioassayed. In addition, plasma samples were assayed by LC/MS to determine the amount of unchanged artelinic acid in the samples. Details of the method of analysis are provided in Appendix B.

The volume of each urine and cage rinse sample was measured. Duplicate aliquots (0.1 mL of urine or 0.5 mL of cage rinse) were placed in oxidizer cones, combusted, and

subsequently radioassayed. The weight of each fecal sample was obtained and individual samples were homogenized in 9 volumes of deionized water. Quadruplicate portions (0.1 or 0.5 mL) of each homogenate were placed in oxidizer cones, combusted, and subsequently radioassayed.

In addition, selected urine and feces samples were analyzed by HPLC to obtain a metabolite profile of the radioactivity in each sample. Urine samples were clarified by centrifugation prior to injection onto the HPLC. Individual fecal homogenates (0.5 g) were alkalinized by mixing with sodium bicarbonate (25 mg) and then centrifuged. The resultant supernatant was analyzed by HPLC. The conditions of analysis of urine and feces were as follows:

Column:	Capcell C8, 4.6 mm × 250 mm (Shiseido Company Ltd., Japan)
Mobile phase A:	0.25% phosphoric acid
Mobile phase B:	50% methanol:20% acetonitrile:30% water containing 0.25% phosphoric acid
Elution:	Gradient

Program:

Min.	Flow mL/min	%A	%B
Initial	1.0	80	20
20	1.0	65	35
40	1.0	55	45
50	1.0	55	45
60	1.0	40	60
70	1.0	0	100
80	1.0	0	100

Detection: In-line radiodetector

Radioactive samples were combusted in a Tri-Carb Sample Oxidizer (Packard Instrument Company, Inc.). Radioanalyses were accomplished in a Tri-Carb 2100TR liquid scintillation analyzer (Packard Instrument Company, Inc.). Radioanalyses were corrected for counting efficiency and background radioactivity.

HPLC analyses were accomplished using a Waters Associates (Milford, MA) system equipped with a Model 510 pump, a Model 710B WISP™ automatic sample injector, a System Gold™ data system and module 168 diode array detector (Beckman Instruments, Inc., San Ramon, CA), and a Flo-One/Beta Series A-500 radio-chromatography detector (Radiomatic Instruments and Chemical Company, Inc.; Meriden, CT).

2.1.2.9 Data Analysis. To calculate the amount of radioactivity present in the total collection of a sample or in a mL of sample, radioassay results were corrected for counting efficiency and background radioactivity and then multiplied by an appropriate factor to correct for sample dilution and/or volume assayed.

The specific activity of each dosing solution was calculated by dividing the radioactivity present in each mL of solution by the concentration, in mg, of artelinic acid in each mL of solution.

Conversion of radioassay results obtained for blood, plasma, urine, and feces to μg equivalents was accomplished by dividing radioactivity amounts in each sample by the specific activity (μCi/mg) of the appropriate dosing solution.

Percent of dose values for urine, cage rinses, and feces were computed by dividing the radioactivity results for individual samples by the amount of radioactivity administered to each animal. Percent of dose values for urinary and fecal metabolites were computed by multiplying the percent of the total radioactivity each metabolite comprised by the total radioactivity in each sample and then dividing by the administered dose.

The above computations were accomplished using Microsoft® Excel software (Microsoft Corporation, Irvine, CA). Where appropriate, mean values and standard deviations were computed using Microsoft® Excel software.

Pharmacokinetic parameters were calculated from blood and plasma concentrations of radioactivity and from plasma concentrations of artelinic acid using a standard computer program (WinNonlin, Standard Edition, Version 1.1; Scientific Consulting, Inc., Apex, NC). Data obtained after IV administration of [^{14}C]artelinic acid were subjected to compartmental analysis and were fit to model 7 (2-compartment model) and model 18 (3-compartment model), and were also subjected to non-compartmental analysis (model 201). Data obtained for dogs dosed PO were subjected to compartmental analysis and were fit to model 3 (1-compartment). For the compartmental analyses, the best fit of the data was determined from Akaike's Information criteria (AIC), Schwartz criteria (SC), the standard error, and graphic fit.

2.1.3 Results

2.1.3.1 Dose Formulation Analysis

The results of the analyses of samples taken from the top, middle, and bottom of the dose formulations of [^{14}C]artelinic acid prepared for iv or po administration indicated that both formulations were homogenous. The mean concentration of artelinic acid in the iv formulation was 9.6 mg/mL, which was 96% of the theoretical concentration. The mean concentration of artelinic acid in the po formulation was 4.6 mg/mL, which was 92% of the theoretical concentration. The mean radioactivity content of the iv formulation was 18.5 μCi/mL and that of the po formulation was 9.3 μCi/mL; both of these values were within 8% of the theoretical value. The radiochemical purity of the iv formulation was determined to be 95.4% and that of the po formulation, 96.1%.

2.1.3.2 Clinical Observations

During the 8 day post-dosing observation period, no adverse reactions or drug-related clinical signs of toxicity were noted for dogs given either an iv or po dose of [^{14}C]artelinic acid.

EKG evaluations which were performed prior to dosing and at 2 and 48 hours after either iv or po administration of [^{14}C]artelinic acid. For one female dog given an iv dose, sinus bradycardia was noted at 2 hours after dosing. No other changes were evident in the EKG tracings recorded from individual dogs at either 2 or 48 hours after either iv or po administration of [^{14}C]artelinic acid.

2.1.3.3 Disposition of [^{14}C]Artelinic Acid Following IV Administration to Dogs

Levels of Radioactivity in Whole Blood and Plasma. The results of the radioanalyses of whole blood collected at various times through 8 days after iv administration of [^{14}C]artelinic acid to two male and two female dogs are shown in Table 1. No differences were discernible in the concentration of radioactivity in whole blood in male and female dogs. At 5 minutes after dosing, peak blood concentrations of radioactivity ranged from 18.6 to 22.9 μg equivalents/mL among the four dosed dogs. The concentrations of radioactivity in whole blood then declined relatively rapidly and were between 2.5 and 3.5 μg equivalents/mL at 2 hours after dosing. Thereafter, the concentration of radioactivity in whole blood declined at a slower rate and at 72 hours after dosing the concentration ranged between 0.22 and 0.29 μg equivalents/mL among the four dogs. Between 72 and 192 hours, the concentration of radioactivity in whole blood declined very slowly, and at 192 hours after dosing the level was between 0.14 and 0.18 μg equivalents/mL among the four dogs.

The corresponding levels of radioactivity in plasma collected from these same dogs are shown in Table 2. At 5 minutes after dosing, the concentration of radioactivity in plasma was higher than the corresponding concentration in whole blood and ranged from 30.3 to 36.4 μg equivalents/mL among the two male and two female dogs. The levels of radioactivity in plasma remained higher than in blood through 24 hours after dosing; thereafter, the levels of radioactivity in plasma approximated the levels in whole blood.

Concentrations of Unchanged Artelinic Acid in Plasma. Plasma concentrations of unchanged artelinic acid in the four dogs administered an iv dose of [^{14}C]artelinic acid are shown in Table 3. At 5 minutes after dosing, the concentration of artelinic acid in plasma ranged from 16.4 to 25.3 μg/mL among the four dosed dogs. For each dog, the concentration of artelinic acid in plasma subsequently declined rapidly and was between 1.0 and 2.6 μg/mL at 1 hour after dosing. Artelinic acid was not detectable in plasma samples collected beyond 4 hours after administration of the iv dose.

During the LC/MS analyses of plasma, only the masses of artelinic acid and the internal standard were monitored; thus, any metabolites of artelinic acid that may have been present in plasma were neither detected nor quantified.

Pharmacokinetic Parameters. Radioactivity data obtained for whole blood following iv administration of [^{14}C]artelinic acid were subjected to both compartmental and non-compartmental analysis; for the compartmental analyses, data were fit to a three compartment model. The results of these analyses are presented in Table 4. From compartmental analyses of the data for the four dogs, the estimated mean half-lives for radioactivity in whole blood were 0.363 hours (range: 0.295 to 0.427 hours), 13.0 hours (10.6 to 17.6 hours), and 308 hours (range: 284 to 376 hours). AUC values (from 0 to infinity) for radioactivity in whole blood ranged from 141 to 187 μg equivalents·hr/mL (mean: 162 μg equivalents/mL). From non-compartmental analysis of the data, the estimated mean terminal half-life of radioactivity was 195 hours (range: 165 to 231 hours) among the four dogs. Computed AUC values (from 0 to infinity) were between 128 and 171 μg equivalents·hr/mL (mean: 141 μg equivalents·hr/mL).

The concentrations of radioactivity in plasma over time in the four dogs given an iv dose of [^{14}C]artelinic acid were subjected to both compartmental and non-compartmental pharmacokinetic analysis. For the compartmental analyses, the data were best fit to a three-compartment model. The results of the compartmental pharmacokinetic analyses are presented in Table 5. From the compartmental analyses, estimated mean half-lives for radioactivity in plasma were 0.33 hours (range: 0.28 to 0.41 hours), 11.5 hours (range: 10.0 to 14.1 hours), and 164 hours (range: 130 to 183 hours). AUC values (from 0 to infinity) among the four dogs ranged from 147 to 205 μg equivalents·hr/mL (mean: 170 μg equivalents·hr/mL). Clearance values for the individual dogs were between 47.1 and 65.7 mL/hr/kg (mean: 57.7 mL/hr/kg). The volume of distribution at steady state ranged from 5753 to 9027 mL/kg. Values derived from non-compartmental analysis of the data are also presented in Table 9. The estimated mean half-life for the terminal phase of elimination was 125 hours (range: 112 to 134 hours) among the four dogs. Computed AUC values (from 0 to infinity) were between 152 and 207 μg equivalents·hr/mL (mean: 170 μg equivalents·hr/mL).

Plasma concentration data for unchanged artelinic acid were subjected to compartmental analysis and the results of this analysis are presented in Table 6. The data were best fit to a two-compartment model. The mean AUC value (from 0 to infinity) for the four dogs given an iv dose of [^{14}C]artelinic acid was 10.8 μg·hr/mL (range: 7.4 to 12.5 μg·hr/mL). Estimated mean half-lives for artelinic acid in plasma were 0.11 and 0.64 hours. Among the four dosed dogs, clearance values ranged from 765 to 1290 mL/hr/kg (mean: 924 mL/hr/kg). The mean volume of distribution at steady state was 545 mL/kg.

Urinary and Fecal Elimination of Radioactivity. Individual data on the urinary and fecal elimination of radioactivity by the two male and two female dogs given an iv dose of approximately 10 mg/kg of [^{14}C]artelinic acid are shown in Table 7. A summary of the data is shown in Table 8. The elimination of radioactivity appeared to be similar for the male and female dogs. Among the individual animals, from 92.3 to 94.5% of the dose was recovered in the combined collections of urine, feces, and cage rinses from 0 to 192 hours after dosing. Of this amount, the majority of the dose was eliminated within the first 24 hours after dosing. Total urinary recoveries of radioactivity during the 192 hour collection interval represented a mean value of 37.2% of the dose. Corresponding mean values for the amount of radioactivity recovered in the cage rinses and feces were 8.8% and 47.3% of the dose, respectively.

Metabolite Profiles of the Radioactivity Eliminated in Urine. Urine samples collected from the individual dogs from 0-12 hours and 12-24 hours after iv administration of [^{14}C]artelinic acid were analyzed by HPLC to obtain metabolite profiles of the radioactivity in the samples; these samples contained the majority of the radioactivity that was recovered in urine.

At least eight radioactive metabolite peaks were resolved in urine obtained 0-12 hours after dosing; no unchanged [^{14}C]artelinic acid was detected (Figure 2). Of the metabolite peaks resolved, the majority of the radioactivity was associated with four peaks having elution times of approximately 15.5, 43.3, 46.0, and 49.3 minutes (Table 9). The percent of the administered dose each metabolite accounted for is presented in Table 10. Among the four dogs, from 1 to 2.6% of the dose was excreted as the 15.5 minute metabolite from 0-12 hours after dosing; from 6.9 to 16.3% as the 43.3 minute metabolite; from 0.9 to 3.9% as the 46 minute metabolite; and from 2.1 to 5.2% as the 49.3 minute metabolite.

The metabolite profile of urine samples collected from 12-24 hours after iv administration of [^{14}C]artelinic acid (Figure 3) was slightly different from that obtained for the urine samples collected from 0-12 hour. The most notable differences in the radioactivity profile of urine were increases in the amount of radioactivity that eluted at 37.5 and 58.5 minutes in the 12-24 hour samples (Table 11). The percent of the administered dose each urinary metabolite accounted for is presented in Table 12. Highest percentages of the dose were associated with the 43.3 minute metabolite, which accounted for from 0.4 to 5.9% of the dose eliminated between 12 and 24 hours, and the 58.5 minute metabolite peak, which accounted for from 1.0 to 2.0% of the dose eliminated during the same interval.

Metabolite Profiles of the Radioactivity Eliminated in Feces. Of the radioactivity eliminated in feces following iv administration of [^{14}C]artelinic acid to dogs, the majority was eliminated in samples collected either 0-12 hours or 12-24 hours after dosing. Feces samples collected at these time points were, thereafter, analyzed by HPLC to obtain metabolite profiles of the radioactivity in the individual samples. Prior to analysis, fecal homogenates (10% in water) were mixed with sodium bicarbonate and extracts were obtained following centrifugation of the samples. Radioanalyses conducted on these fecal extracts indicated that this method of fecal preparation resulted in the extraction of a mean value of 64.6% of the radioactivity in the individual fecal samples. From previous experience, this extraction efficiency was considered to be acceptable and relatively high for this biological matrix.

A representative radiochromatogram obtained during the HPLC analyses of feces collected 0-12 hour after iv administration of [^{14}C]artelinic acid is shown in Figure 4. Radiochromatograms obtained for feces samples collected 12-24 hours after dosing were qualitatively similar to the profile obtained for samples collected at 0-12 hours. The majority of the radioactivity in feces was accounted for by one major and two minor metabolite peaks that eluted at retention times of approximately 60, 76, and 79 minutes; no unchanged artelinic acid was detectable in feces (Table 13). The percent of the administered dose each metabolite accounted for is presented in Table 14. The highest percentage of the dose was associated with

79 minute metabolite which, in feces collected from 12-24 hours after dosing, accounted for approximately 4 to 13% of the administered dose.

2.1.3.4 Disposition of [^{14}C]Artelinic Acid Following PO Administration to Dogs

Levels of Radioactivity in Whole Blood and Plasma. The results of the radioanalyses of whole blood collected at various times through 8 days after po administration of approximately 10 mg/kg of [^{14}C]artelinic acid to two male and two female dogs are shown in Table 15. Among the individual dogs, peak concentrations of radioactivity in whole blood were observed at 30 minutes after dosing (earliest time point) and ranged from 5.1 to 7.5 μg equivalents/mL. Subsequently, in each dog, the level of radioactivity in whole blood declined and was between 0.22 and 0.34 μg equivalents/mL at 72 hours after dosing. Thereafter, the concentration of radioactivity in whole blood decreased very slowly and at 192 hours was between 0.13 and 0.18 μg equivalents/mL among the four dosed dogs.

The corresponding levels of radioactivity in plasma collected from these same dogs are shown in Table 16. At 30 minutes after dosing, the concentrations of radioactivity in plasma were higher than the corresponding concentrations in whole blood and ranged from 8.5 to 12.3 μg equivalents/mL among the two male and two female dogs. As observed after administration of the iv dose, the concentration of radioactivity in plasma was higher than the corresponding concentration in whole blood through 24 hours. Beyond this time, the levels of radioactivity in plasma approximated the levels in whole blood.

Concentrations of Unchanged Artelinic Acid in Plasma. Concentrations of artelinic acid in plasma collected at various times after po administration of [^{14}C]artelinic acid to the two male and two female dogs are shown in Table 17. Peak concentrations of artelinic acid observed in plasma at 30 minutes to 1 hour after dosing ranged from 3.1 to 4.2 μg/mL. Thereafter, in each dog, the concentration of artelinic acid in plasma decreased and was between 1.3 and 2.8 μg/mL at 2 hours after dosing. With one exception, unchanged artelinic acid was not detectable in plasma samples collected beyond 4 hours after administration of the po dose.

As noted above, only the masses of artelinic acid and the internal standard were monitored during the LC/MS analyses of plasma; thus, any metabolites of artelinic acid that may have been present in plasma were not detected and not quantified.

Pharmacokinetic Parameters. Radioactivity data obtained for whole blood following po administration of [^{14}C]artelinic acid were subjected to non-compartmental analysis; the data were not well fit to a pharmacokinetic model. The results of these analyses are presented in Table 18. The estimated half-life for the terminal elimination of radioactivity from whole blood ranged from 156 to 185 hours (mean: 168 hours) among the four dosed dogs. AUC values (from 0 to infinity) were between 103 and 134 μg equivalents·hr/mL (mean: 120 μg equivalents·hr/mL). Based on the mean AUC values computed from the concentrations of radioactivity in whole blood over time, the estimated mean value for the oral bioavailability of radioactivity derived from [^{14}C]artelinic acid was 85% for the four dosed dogs.

Plasma concentrations of radioactivity over time after administration of [^{14}C]artelinic acid were also not well fit to a kinetic model. The data were subjected to non-compartmental analysis and the results of these analyses are also presented in Table 18. The estimated half-life for the terminal elimination of radioactivity from plasma ranged from 99 to 144 hours (mean: 129 hours) among the four dosed dogs. AUC values (from 0 to infinity) were between 114 and 178 μg equivalents·hr/mL (mean: 150 μg equivalents·hr/mL). Based on mean AUC values calculated from concentrations of radioactivity in plasma over time, the estimated mean oral bioavailability of radioactivity derived from [^{14}C]artelinic acid was 88% for the four dosed dogs.

Plasma concentration data for unchanged artelinic acid were subjected to compartmental analysis and the results are presented in Table 6. The data were best fit to a one-compartment model. The mean AUC value (from 0 to infinity) was 7.0 μg·hr/mL (range: 5.4 to 9.5 μg·hr/mL). The estimated mean half-life for artelinic acid in plasma was 0.73 hours. Based on the mean AUC values for unchanged artelinic acid computed after iv and po administration, the mean oral bioavailability of artelinic acid was estimated to be 67% in the four dosed dogs.

Urinary and Fecal Elimination of Radioactivity. Individual data on the urinary and fecal elimination of radioactivity by the two male and two female dogs given a po dose of approximately 10 mg/kg of [^{14}C]artelinic acid are shown in Table 19. A summary of the data is presented in Table 8. The elimination of radioactivity appeared to be similar for the male and female dogs. Among the individual animals, from 90.8 to 94.3% of the dose was recovered in the combined collections of urine, feces, and the cage rinses from 0 to 192 hours after dosing. Of this amount, the majority was eliminated within the first 48 hours after dosing. Total urinary recoveries of radioactivity for the four dogs during the 192 hour collection interval represented a mean value of 25.5% of the dose. Corresponding mean values for the amount of radioactivity recovered in the cage rinses and feces were 9.9% and 57.5%, respectively.

Metabolite Profiles of the Radioactivity Eliminated in Urine. Urine samples collected from the individual dogs from 0-12 hours and 12-24 hours after po administration of [^{14}C]artelinic acid were analyzed by HPLC to obtain metabolite profiles of the radioactivity in the samples; these samples contained the majority of the radioactivity that was eliminated in urine after po dosing.

The metabolite profiles of urine samples collected from either 0-12 hours or 12-24 hours after po administration of [^{14}C]artelinic acid were qualitatively similar to the corresponding urinary metabolite profiles observed after iv administration of the compound (Tables 9 and 11). The percent of the administered dose each metabolite accounted for is presented in Tables 10 and 12. In urine collected from individual dogs 0-12 hours after po dosing, the highest percentages of the dose were associated with the 43.3 minute metabolite which accounted for as much as 11.7% of the dose, and with the 49.3 minute metabolite which accounted for as much as 3.1% of the dose. In urine collected from 12-24 hours after po dosing, the highest percentages of the dose were associated with the 43.3 and 58.5 minute metabolite peaks which accounted for 1.0% to 4.9% and 0.6% to 2.7% of the dose, respectively.

Metabolite Profiles of the Radioactivity Eliminated in Feces. The radioactivity profile of feces samples collected after po administration of [^{14}C]artelinic acid was qualitatively similar

to the profile obtained after iv administration of the compound. The majority of the radioactivity in feces was accounted for by one major and two minor metabolite peaks with elution times of 60, 76, and 79 minutes; no unchanged artelinic acid was detected (Table 13). The percent of the administered dose each metabolite accounted for in feces collected from 0-12 or 12-24 hours after po dosing is presented in Table 14. During this collection interval, the percent of the dose accounted for by the 60, 76, and 79 minute metabolites was between <0.1% to 3.1 %, 6.6% to 7.8%, and 8.1% to 13.0%, respectively.

2.1.3.5 Metabolite Analyses

Based in part on work accomplished by Maggs and coworkers,[7] it was predicted that the metabolites of artelinic acid might include various glucuronides of the free acid and hydroxylated derivatives. Following this approach, a Perkin Elmer Sciex API 3000 LC/MS/MS instrument was used to screen urine samples for expected metabolites of artelinic acid by neutral loss, by scanning in single quadrupole mode, and by mixed reaction monitoring.

Utilizing the above methods, several urinary metabolites of artelinic acid were tentatively identified. The identifications were based on adding mass units, fragmentation patterns, and literature on the known metabolites of artelinic acid and related compounds. The metabolites tentatively identified included the following:

·Glucuronide of DQHS: A urine sample collected from a dog dosed with artelinic acid was injected into an Aquasil C18 guard column on the LC/MS/MS instrument and the effluent was monitored for the product ions of m/z=459 in the negative mode. M/z=459 would correspond to m/z=283 (the DQHS negative ion) + glucuronide (m/z=176). The actual ion products observed were m/z=283 and m/z=193 (Figure 5). The m/z=193 corresponded to the open form of the glucose molecule and suggested the molecule had been conjugated to a hydroxyl. In the positive ion mode, the expected ion was m/z=499, which would correspond to the K salt of the glucuronide. The product ion observed was m/z=323, which corresponded to DQHS + K (284+ 39). Based on these and other data, the major urinary metabolite of artelinic acid was identified as a glucuronide of DQHS.

·Glucuronides of hydroxy DQHS: A urine sample collected from a dog dosed with artelinic acid was injected into an Aquasil C18 guard column on the LC/MS/MS instrument and the effluent was monitored for the product ions of m/z=475 in the negative mode. M/z=475 would correspond to m/z=283 (DQHS) + 16 (oxygen) + 176 (glucuronide). The product ions observed were m/z=193 (open glucose), m/z=299 (hydroxy DQHS), and a trace of m/z=175 (Figure 6). The position of the hydroxyl group could not be assigned based on this fragmentation. As shown in Figure 7, an additional metabolite, having a slightly different retention time (3.34 minutes, instead of 2.90 minutes), was found to yield the same fragmentation, producing m/z=193, m/z=113, and m/z=175 fragments. This fragmentation pattern suggested that one metabolite was the result of conjugation on the hydroxy group added to the ring and the other metabolite was the result of conjugation of the hydroxy group of DQHS.

·Glucuronide of artelinic acid: A urine sample collected from a dog dosed with artelinic acid was injected into an Aquasil C18 guard column on the LC/MS/MS instrument and the effluent

was monitored for the product ions of m/z=593 in the negative mode. M/z=593 corresponded to the negative ion of artelinic acid (m/z=417) + glucuronide (m/z=175). It was proposed that glucuronide conjugation would occur on the carboxylic acid group of artelinic acid, thus, no m/z=193 was expected for the open glucose ion. The actual ion products observed were m/z=417 (artelinic acid negative ion), m/z=175 (glucuronide), and 113 (a fragment associated with glucuronides) (Figure 8).

·Glucuronide of hydroxy artelinic acid: A urine sample collected from a dog dosed with artelinic acid was injected into an Aquasil C18 guard column on the LC/MS/MS and the effluent was monitored for the product ions of m/z=609 in the negative mode. M/z=609 would correspond to the negative ion of artelinic acid (m/z=417) + oxygen (16) + glucuronide (176). The actual products observed were m/z=433 and m/z=175 (Figure 9). These corresponded to 417 + 16 = 433 (hydroxylated artelinic acid) and 175 (glucuronide closed ring). The absence of m/z=193 suggested the conjugation occurred on the carboxylate group of artelinic acid.

·Possible sulfate conjugate: A metabolite was observed in urine using a product ion scan of m/z=514 in the negative mode. The product ions observed were m/z=212, m/z=301, and m/z=434. These data suggested that the metabolite was a sulfate conjugate of artelinic acid. M/z=514 would correspond to 418 (artelinic acid with no charge) + 80 (sulfate with negative charge) + 16 (oxygen). The m/z=434 would correspond to the hydroxylated artelinic acid fragment.

2.2 Task Order SR98-3: Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

2.2.1 Objective

The objective of this study was to determine the dose-related pharmacodynamic effects of artelinic acid, as assessed by signs of clinical and pathological toxicity, in rats given daily oral gavage doses for 14 consecutive days. This was to include determination of a no-effect dose level, target organ toxicity, and the reversibility of target organ toxicity. The study was initially designed to entail two phases: an initial dose range finding evaluation to select an appropriate dose(s) of artelinic acid that produced a measurable pharmacodynamic effect; and a subsequent definitive study to fully characterize any dose-related pharmacodynamic effects of artelinic acid. In each phase of the study, a positive control group, that was to receive arteether, was included. Parameters evaluated included: clinical signs of toxicity, body weights, and histopathological evaluations of tissues. Upon completion of the pilot range finding phase of this study, the Artelinic Acid Development team decided to pursue the development of an injectable formulation of artelinic acid. For this reason, the original plans to conduct a definitive study with orally administered artelinic acid in rats were terminated and only the pilot phase of the study was completed. The results of this pilot phase are reported herein.

2.2.2 Materials and Methods

2.2.2.1 Test System. Male and female Sprague-Dawley rats (CD®; Charles River Laboratories; Raleigh, NC) were used. Animals were quarantined for approximately 1 week prior to the start of the study. During the quarantine and study periods, the rats were individually housed in solid bottom, polycarbonate cages, lined with hardwood chip bedding. The cages were maintained on stainless steel racks with automatic watering systems. Animal care and housing were in compliance with the standard operating procedures (SOPs) of Southern Research, the *Guide for the Care and Use of Laboratory Animals*, 7th edition[6] and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198). Food (Purina Certified Rodent Diet #5002; PMI Feeds, Inc.; St. Louis, MO) and water (Birmingham municipal supply) were available to all rats *ad libitum*. The animals were maintained in an environmentally-controlled room in which temperature and humidity were monitored daily. Room lights were controlled by an automatic timer set to provide 12 hours of light (0600-1800 hours CST) and 12 hours of dark each day. The rats were approximately 9 weeks old on Day 1 of the study.

2.2.2.2 Test Articles. One shipment (two bottles) containing a total of 312 grams of artelinic acid (WR255663; Bottle No. BP12419; manufacturer's code NJ33-113-1) was supplied by Walter Reed Army Institute of Research (WRAIR; Washington, D.C.) and received at Southern Research on 8/11/99. Twenty (20) grams of arteether (WR 255131; Bottle No. BL48816; manufacturer's code FJ18-97-3) were also supplied by WRAIR and received at Southern Research on 9/24/99. The bulk quantities of artelinic acid and arteether were stored frozen (-20°C) from the time of receipt until the time of use (8/23-30/99) and were assumed to be stable when so stored.

2.2.2.3 Vehicles. The vehicles used for preparation of the artelinic acid dose formulations were sterile water, USP (Phoenix Pharmaceutical, Inc.; St. Joseph, MO; Lot 8111186, expiration date November 2001), polyoxyethylene sorbitan monooleate (Tween 80; Sigma Chemical Company; St. Louis, MO; Lot 87H0648; Southern Research assigned expiration date January 31, 2001), and carboxymethylcellulose (CMC; Sigma Chemical Company; St. Louis, MO; Lot 126H0367; Southern Research assigned expiration date January 31, 2001). The vehicle used for preparation of the arteether dose formulation was sesame oil (Sigma Chemical Company; St. Louis, MO; Lot 48H0242; Southern Research assigned expiration date January 31, 2001).

2.2.2.4 Dose Formulation Preparation. Prior to preparation of individual dose formulations of artelinic acid, a solution containing 1% (w/v) CMC and 0.2% (v/v) Tween 80 was prepared.

Artelinic acid dose formulations were prepared by weighing the required amount of artelinic acid into a glass container and adding the appropriate volume of 1% CMC: 0.2% Tween 80. The formulations were homogenized using a Potter-Elveham-type homogenizer until the artelinic acid was uniformly suspended (visual inspection) in the vehicle. Dose formulations of artelinic acid were prepared to contain artelinic acid at concentrations of 2, 4, 8, 16, or 64 mg/mL. The corresponding vehicle control formulation consisted of 1% (w/v) CMC and 0.2% (v/v) Tween 80.

Individual dose formulations of artelinic acid and the corresponding vehicle control formulation were prepared twice during the study and each formulation was used for dosing with 9 days of preparation. The dose formulations were stored at room temperature.

The arteether dose formulation was prepared by adding arteether to an appropriate volume of sesame oil and sonicating and/or stirring until well mixed. The dose formulation of arteether was prepared to have an arteether concentration of 25 mg/mL. The dose formulation of arteether was stored at room temperature. Previous investigations have shown that arteether is stable in sesame oil for at least 1 year when maintained at room temperature.[8]

2.2.2.5 Dose Formulation Analysis. Upon preparation, each artelinic acid and arteether dose formulation was analyzed by HPLC for chemical concentration and homogeneity. In addition, to assess stability, the dose formulations of artelinic acid were also analyzed at the end of the period of use. Dose formulations of artelinic acid were analyzed under the HPLC conditions described in Section 2.1.2.3. Dose formulations of arteether were analyzed under the following HPLC conditions:

Column: Bondapak C_{18}, 10 μm, 300 mm x 3.9 mm (Phenomenex; Torrance, CA)
Mobile Phase: 60% Acetonitrile:40% water
Elution: Isocratic
Temperature: Ambient
Flow rate: 1.5 mL/min
Detection: UV absorbance at 216 nm

2.2.2.6 Group Assignment and Dose Procedure. Forty four rats were each assigned to one of five artelinic acid treated groups (4 rats/sex/treatment group for Groups A-D and 2 rats/sex in Group E) or to one of two control groups [2 rats/sex/treatment group in either the vehicle control group (negative control; Group V) or the arteether group (positive control; Group F)]. All animals in Groups V and A-E received oral (gavage) doses of vehicle or artelinic acid once each day for 14 days; the dose volume given was 5 mL/kg/day. The rats in Group F received intramuscular injections of arteether once each day for 14 days; the dose volume given was 0.5 mL/kg/day. Each animal was dosed in the morning, at approximately the same time, each day. Animal assignment was as follows:

Group	Compound	Route	Dose Level (mg/kg/day)	Number of Animals	
				Male	Female
V	None[a]	PO	0	2	2
A	Artelinic acid	PO	10	4	4
B	Artelinic acid	PO	20	4	4
C	Artelinic acid	PO	40	4	4
D	Artelinic acid	PO	80	4	4
E	Artelinic acid	PO	320	2	2
F	Arteether	IM	12.5	2	2

[a]Rats were given 1% CMC:0.2% Tween 80

2.2.2.7 Clinical Observations. All animals were checked twice daily for signs of mortality/moribundity. On Days 1-14, a detailed clinical examination of each animal was performed within approximately 1 hour after dosing. Additional cage-side observations (for appearance, gait, activity, level of arousal, and motor coordination) were performed on each animal 1-3 hours and 7-9 hours after dosing; any changes from normal behavior or activity were documented. On Days 15-20, animals were assessed (cage-side observations) twice a day for appearance, gait, activity, level of arousal, and motor coordination, and any changes from normal behavior or activity were documented. The technicians who made the observations and recorded the clinical signs were "blinded" to the treatment.

2.2.2.8 Body Weights. Individual body weights were recorded on Days 1-14 and prior to sacrifice (Day 21).

2.2.2.9 Clinical Pathology. Hematological and clinical chemistry evaluations were performed on animals on Day 21, prior to necropsy. Rats were fasted overnight with access to water. Rats were anesthetized with CO_2/O_2 and blood samples were obtained from the retro-orbital plexus into tubes containing EDTA (hematology evaluations) or into tubes with no anticoagulant (clinical chemistry evaluations). The samples were used for the following determinations:

Hematology:

Hemoglobin (HGB)
Hematocrit (HCT)
Total leukocyte count (WBC)
Erythrocyte count (RBC)
Platelet count (PLT)
Reticulocyte counts (RETIC)
Differential leukocyte count
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)

Clinical Chemistry:

Glucose (GLUC)
Creatinine (CREA)
Serum aspartate aminotransferase (AST)
Sodium (Na)
Potassium (K)
Chloride (Cl)
Blood urea nitrogen (BUN)
Serum alanine aminotransferase (ALT)
Alkaline phosphatase (ALP)
Total protein (TP)
Albumin (ALB)
Blood urea nitrogen/creatinine ratio (BUN/CRE ratio)

2.2.2.10 Necropsy and Histopathology. At sacrifice on Day 21, individual animals were anesthetized with pentobarbital (40-50 mg/kg, ip) and then exsanguinated with a flush solution of normal (0.9%) saline containing 1 unit of heparin per mL. Immediately following the heparinized saline flush, animals were perfused with a fixative of 1% glutaraldehyde and 4% paraformaldehyde buffered in a phosphate buffer. After fixation was complete, the central nervous system of each animal was removed for neurotoxicological evaluation.

For the neurotoxicological evaluation, tissues were embedded in paraffin and sectioned (5 μm). The entire hind brain was sectioned, mounted, and every 20 to 23rd section was stained with hematoxylin and eosin (H&E), Luxol Fast Blue with cresyl violet. Individual slides were evaluated by a pathologist at Southern Research Institute and lesions were graded on a five point scale with 0 as normal and 4 as the most severe. The grading scale used was as follows:

Trace +1, one or two degenerative or necrotic neurons present in one brain nucleus;

Mild +2, three or more degenerative or necrotic cells present in one brain nucleus or one or two degenerative cells in two nuclei of the brain;

Moderate +3, more than half of the neurons in a nucleus showing degenerative or necrotic changes or 3 or more degenerative neurons in 3 or more brain nuclei;

Severe +4, most or all of the neurons in a nucleus necrotic with a cellular response to the necrosis or many degenerative and necrotic neurons in numerous brain nuclei with evidence of cellular response to the necrosis.

Selected histopathology slides were also evaluated by a pathologist (Dr. James Petras) at WRAIR.

2.2.2.11 Statistical Analyses. In that this was a preliminary evaluation and only a limited number of animals were included in each dose group, no statistical analyses of the data were performed.

2.2.3 Results

2.2.3.1 Dose Formulation Analysis

The results of the concentration/homogeneity analyses of the dose formulations of artelinic acid are presented in Table 20; for the homogeneity determinations, samples for analysis were removed from the top, middle, and bottom of each formulation. The mean concentrations of artelinic acid determined prior to or at the beginning of the period of usage of individual formulations ranged from 74% to 97% of the theoretical concentrations. The relative standard deviation (RSD) values for the analyses of the top, middle, and bottom samples ranged from 1.2% to 19%. The post-dose analyses showed results of 74% to 99% of the theoretical concentrations. For formulations prepared for the first week of dosing, the concentrations of artelinic acid were also determined after each formulation had been distributed to individual

bottles for use in the animal rooms (i.e., six bottles for each concentration level). The results of analyses of samples removed from each bottle were consistent with the initial analyses of the top, middle, and bottom samples and indicated the mean concentration of artelinic acid in each formulation was between 78% and 94% of the theoretical concentration.

These results of the analyses of the dose formulations indicated that the measured concentration of artelinic acid in most of the formulations was inconsistent with the expected (theoretical) concentration and lower than the usual normal limit (±10% theoretical). The amount of artelinic acid used to prepare each formulation was verified by a check of the analytical balance records, and the final volume of each dose formulation was double-checked to assure the proper amount of vehicle had been used. The standards prepared for the standard curve were reproducible, indicating that the analytical method was not problematic. Thus, the discrepancy in concentrations could have resulted from improper mixing of the artelinic acid formulations or incorrect sampling of the formulations. In most cases, the %RSD for replicate samples removed from each formulation was <5%, which suggested that the mixing procedure was adequate to produce uniform suspensions. The aliquots that were removed from each formulation for analysis were weighed to verify the volume. Due to the limited supply of artelinic acid on-hand, the decision was made to use the formulations without verification of correct test article concentration. In making this decision, the Study Director proceeded on the assumption that the formulations had been made correctly, and the problems with verification of the test article concentration were in some way associated with the analytical procedures.

The dose formulation of arteether used during the study was also analyzed prior to use for homogeneity and chemical concentration. The data indicated the measured concentration of arteether in the formulation 24.9 mg/mL, which was 99% of the theoretical concentration. The RSD value of 0.83% for the analyses of samples removed from the top, middle, and bottom indicated the formulation was homogeneous.

2.2.3.2 Mortality

One of two female rats in the 320 mg/kg/day artelinic acid dose group was found dead on Day 10 of the dose period; the other female rat in this dose group was found dead on Day 12 of the dose period. The remaining animals on the study survived until their scheduled sacrifice on Day 21.

2.2.3.3 Clinical Observations

Clinical observations are summarized in Table 21. No clinical signs of toxicity were observed for male or female rats in the 10, 20, 40, or 80 mg/kg/day artelinic acid dose groups or the 12.5 mg/kg/day arteether dose group.

Drug-related clinical signs of toxicity were noted only for the male and female rats given 320 mg/kg/day of artelinic acid. Male rats in this dose group were emaciated between Days 7 and 21; other adverse clinical signs noted for these animals between Days 15 and 18 included dehydration, an eye discharge, hypoactivity, and tics. An eye discharge (Day 9) and emaciation (Days 8-11) were noted for the female rats in this dose group prior to death on Day 10 or 12.

2.2.3.4 Body Weights

A summary of the mean body weight data is presented in Table 22. Male rats in all treatment groups, except those in the 320 mg/kg/day artelinic acid dose group, gained comparable amounts of body weight to the male rats in the (negative) vehicle control group. Male rats given daily oral doses of 320 mg/kg/day of artelinic acid lost weight between Days 3 and 21; on Day 21, the average body weight of male rats in the 320 mg/kg/day dose group was 55% of the average body weight of male rats in the (negative) vehicle control group. Female rats given 10, 20, 40, or 80 mg/kg/day of artelinic acid gained less weight between Day 1 and the day of sacrifice than did female rats in the vehicle control group; there appeared to be a dose-related reduction in the body weight gain of female rats in these four artelinic acid dose groups. Female rats in the 320 mg/kg/day dose group lost weight between Day 1 and the day of death (Day 10 or 12); on the day prior to death, both of these rats weighed approximately 60% of the average body weight of the female rats in the vehicle control group.

While daily i.m. administration of 12.5 mg/kg/day of arteether had no apparent effect on the body weights of male rats, it caused an apparent reduction in the rate of body weight gain in female rats.

2.2.3.5 Hematology

A summary of the hematology data is presented in Table 23. Potentially drug-related changes in hematology parameters that were observed on Day 21 and considered to be biologically relevant included mild increases in mean reticulocyte counts and erythrocyte MCV values. Biologically relevant changes were defined as between-group differences in group mean or individual values that were not consistent with expected intraindividual, interindividual, or analytical variability.

Group mean reticulocyte counts of male rats in the 20, 40, 80, and 320 mg/kg/day artelinic acid dose groups were 178%, 189%, 193%, and 319%, respectively, of the group mean reticulocyte values observed for male rats in the vehicle control group. Group mean reticulocyte counts observed for female rats in the 20, 40, and 80 mg/kg/day dose groups were 232%, 226%, and 316%, respectively, of the group mean reticulocyte count of female rats in the vehicle control group. (Note: the two female rats in the 320 mg/kg/day dose group died prior to the scheduled hematological evaluation on Day 21). A mild but potentially biologically relevant increase in erythrocyte MCV values was observed in male rats in the 20 and 80 mg/kg/day artelinic acid dose groups and in female rats in the 80 mg/kg/day dose group. MCV values that were observed for male and female rats in these dose groups ranged from 108%, 110%, and 109% of the values observed for rats in the corresponding vehicle control groups. Concurrent minimal decreases in group mean RBC values, but not HGB, or HCT values, were observed only for male and female rats in the 80 mg/kg/day dose group. No appreciable decreases in RBC values were observed for rats in other dose groups. No dose-related trends were observed for RBC or MCV values. A majority or at least half of the individual rats in the 10 (female only), 20, 40, 80, and 320 (male only, no females available for analysis) mg/kg/day dose groups were observed to have reticulocyte counts that were greater than the range of values (mean ± 2 s.d.) reported for historical data. Due to the consistency of the finding of increased reticulocyte

counts, the increased incidence of reticulocytosis, and the presence of a dose relationship for male rats in the 80 and 320 mg/kg/day dose groups and female rats in the 40 and 80 mg/kg/day dose groups, this finding was considered to be drug related. Without concurrent anemia, the toxicological relevance of this finding was uncertain. The between group variability observed in WBC and differential leukocyte counts in this study were considered to reflect normal biological variability and not drug- or treatment-related changes.

2.2.3.6 Clinical Chemistry

A summary of the clinical chemistry data is presented in Table 24. The only potentially drug-related change in clinical chemistry parameters was a biologically relevant decrease in mean ALP activity.

The group mean ALP value that was observed for male rats in the 320 mg/kg/day artelinic acid dose group was 49% of the group mean ALP value observed for male rats in the vehicle control group. A trend towards decreased ALP activity was observed in all male rats given daily oral doses of artelinic acid. The decreased ALP activity observed for male rats in the 320 mg/kg/day dose group was probably secondary to decreased food intake and body weight loss noted for rats in this group.

Minimal increases observed in AST activity (<150% of vehicle control value) in male rats in the 20 and 320 mg/kg/day dose groups and in glucose concentration (<140% of the vehicle control value) observed for male rats in the 320 mg/kg/day dose group were considered to be nonspecific changes and were not considered to be drug related.

2.2.3.7 Histopathology

Histopathological findings for brain, as evaluated by the pathologist at Southern Research, are listed in Table 25. "Normal" indicates that all sections of the hind brain were within normal limits. All observations of "neuron degeneration" were for the trapezoid nucleus, except where noted. The neuron degeneration was characterized by loss of Nissl staining, swelling, and margination of the nucleus. These cells appeared to have red/brown cytoplasm on H&E section. More severe lesions had clumping of eosinophilic debris in some cells, minimal evidence of satellitosis, and minimal gliosis.

As shown in Table 25, neuropathological lesions were present in rats given the positive control agent, arteether, and in rats given artelinic acid at doses of 40 mg/kg/day and above. At artelinic acid doses of 40 and 80 mg/kg/day, the lesions consisted of neuronal degeneration of the trapezoid nucleus. At an artelinic acid dose of 320 mg/kg/day, the lesions observed for male rats included neuronal degeneration of the trapezoid nucleus and multiple nuclei in the hind brain; due to mortality, the female rats in the 320 mg/kg/day dose group were not perfused and reliable neurohistopathological evaluations of these animals could not be obtained.

The peer review evaluation of these histopathological slides, which was conducted by Dr. James Petras at WRAIR, was in agreement with these findings.

2.3 Task Order SR99-1: Effect of Artelinic Acid on Dogs after Oral Administration for 14 Days

2.3.1 Objective

The objective of the study described herein was to determine the dose-related pharmacodynamic effects of artelinic acid, as assessed by signs of clinical and pathological toxicity, in dogs following daily oral administration for 14 consecutive days. This included determination of a no-effect dose level, target organ toxicity, and the reversibility of target organ toxicity. The study was initially designed to be conducted in two phases consisting of an initial pilot dose range finding evaluation to select an appropriate dose(s) of artelinic acid that produced a measurable pharmacodynamic effect and a subsequent definitive study to fully characterize any dose-related pharmacodynamic effects of artelinic acid. In each phase of the study, a positive control group, that was to receive arteether, was to be included. Parameters to be evaluated included: examinations of individual animals for clinical signs of toxicity, body weight measurements, and histopathological evaluations of tissues. Upon completion of the pilot range finding phase of this study, the Artelinic Acid Development team decided to pursue the development of an injectable formulation of artelinic acid. For this reason, the original plans to conduct a definitive study with orally administered artelinic acid in dogs were terminated and only the pilot phase of the study was completed. The results of this pilot phase are reported herein.

2.3.2 Material and Methods

2.3.2.1 Test System. Twelve (12) male and 12 female dogs (Marshall Farms, Inc.; South Rose, NY) were used in the study. The dogs arrived at Southern Research Institute (Southern Research) on September 10, 1999. The dogs were quarantined for approximately 3 weeks prior to the start of the study and were between 8 and 9 months old on Day 1.

During the quarantine and study periods, the dogs were individually housed in stainless steel cages. Animal care and housing were in compliance with the standard operating procedures (SOPs) of Southern Research, the *Guide for the Care and Use of Laboratory Animals*, 7th edition[6] and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198). Each dog was allowed access to Certified Canine Diet #5007 (PMI Feeds, Inc.; St. Louis, MO) for approximately 2 hours each day. Tap water (Birmingham municipal supply) was available to each animal *ad libitum*. No known contaminants were present in the food or water that would affect the study outcome. The animals were maintained in an environmentally-controlled room in which temperature and humidity were monitored daily. Environmental controls were set to maintain temperature and humidity ranges between 64.0-84.0 °F and 30.0-70.0%; excursions did not affect the health of the animals or the outcome of the study. Room lights were controlled by an automatic timer set to provide 12 hours of light (0600-1800 hours CST) and 12 hours of dark each day.

2.3.2.2 Test Articles. One shipment (two bottles) containing a total of 312 grams of artelinic acid (WR 255663; Bottle No. BP12419; manufacturer's code NJ33-113-1; expiration date not provided; Southern Research Lots D78/L-1 and D78/L-2) was supplied by Walter Reed

Army Institute of Research (WRAIR), Washington, D.C. and received at Southern Research on August 11, 1999. Twenty (20) grams of arteether (WR 255131; Bottle No. BL48816; manufacturer's code FJ18-97-3; expiration date not provided; no Southern Research lot number assigned) were also supplied by WRAIR and received at Southern Research on September 24, 1999. The bulk quantities of artelinic acid and arteether were stored frozen (–20 °C) from the time of receipt until the time of use (October 4-11, 1999) and were assumed to be stable when so stored.

2.3.2.3 Vehicles. The reagents used for preparation of the artelinic acid dose formulations were deionized water; polyoxyethylene sorbitan monooleate (Tween 80; Lot 87H0648; Southern Research-assigned expiration date January 31, 2001; Sigma Chemical Company; St. Louis, MO); and carboxymethylcellulose, sodium salt, medium viscosity (CMC; Lots 126H0367 and 108H0052; Southern Research-assigned expiration date January 31, 2001, for each lot; Sigma Chemical Company; St. Louis, MO). Prior to preparation of individual dose formulations of artelinic acid, a solution containing 1% (w/v) CMC and 0.2% (v/v) Tween 80 was prepared in deionized water. Dose formulations of arteether were prepared in sesame oil (Lot 48H0242; Southern Research-assigned expiration date January 31, 2001; Sigma Chemical Company; St. Louis, MO).

2.3.2.4 Dose Formulation Preparation. Artelinic acid dose formulations were prepared by weighing the required amount of artelinic acid into a glass container and adding the appropriate volume of 1% CMC: 0.2% Tween 80. The formulations were homogenized using a Potter-Elveham-type homogenizer until the artelinic acid was uniformly suspended (visual inspection) in the vehicle. Dose formulations of artelinic acid were prepared to contain artelinic acid at concentrations of 2, 4, 8, and 32 mg/mL of artelinic acid. The corresponding vehicle control formulation consisted of 1% CMC:0.2% Tween 80.

The individual dose formulations of artelinic acid and the corresponding vehicle were prepared twice during the study and each formulation was used for dosing within 8 days of preparation. The dose formulations were maintained at room temperature. Dose formulations of artelinic acid in CMC:Tween 80 have been shown to be stable for at least 7 days when maintained at room temperature.[9]

The arteether formulations were prepared by adding a weighed quantity of arteether to an appropriate volume of sesame oil and sonicating and/or stirring until well mixed. The dose formulation of arteether was prepared to have an arteether concentration of 100 mg/mL. One dose formulation of arteether was prepared and used for all doses. The dose formulation of arteether was maintained at room temperature. Previous investigations have shown that arteether is stable in sesame oil at room temperature for at least 1 year.[8]

2.3.2.5 Dose Formulation Analysis. Each artelinic acid and arteether dose formulation was analyzed by HPLC for chemical concentration and homogeneity. Dose formulations of artelinic acid were analyzed under the conditions described in Section 2.1.2.3. Dose formulations of arteether were analyzed under the conditions described in Section 2.2.2.5.

2.3.2.6 Group Assignment and Dosing. Twenty four dogs were each assigned to one of four test article treated groups (two dogs/sex/treatment group for Groups A-D) or to one of two control groups [two dogs/sex/treatment group in either the vehicle control group (negative control; Group V) or the arteether group (positive control; Group E)]. All animals in Groups V and A-D received oral (gavage) doses of vehicle or artelinic acid once each day for 14 consecutive days, while the dogs in Group E received intramuscular (im) injections of arteether once each day for 14 consecutive days. Animal assignment was as follows:

Group	Compound	Dose		Number of Animals	
		Route	Volume (mg/kg/day)	Males	Females
V	None[a]	PO	0	2	2
A	Artelinic acid	PO	20	2	2
B	Artelinic acid	PO	40	2	2
C	Artelinic acid	PO	80	2	2
D	Artelinic acid	PO	320	2	2
E	Arteether	IM	20	2	2

[a] Dogs were given 1% CMC:0.2% Tween 80

2.3.2.7 Clinical Observations. On Days 1-21, dogs were observed at least twice daily for mortality/morbundity. On Days 1-14, a detailed clinical examination of each animal was performed within approximately 1-3 hours after dosing. In addition, on Days 1-14, animals were carefully assessed two additional times per day (with a minimum of 4 hours between each evaluation) for signs of central nervous system toxicity; posture, activity, level of arousal, gait, breathing, and motor coordination were assessed, and any changes from normal behavior or activity were documented. The technicians who made the observations and recorded the clinical signs were "blinded" to the treatment.

2.3.2.8 Body Weights. Body weights were obtained daily on Days 1-14 and prior to necropsy on Day 15 or Day 21.

2.3.2.9 Clinical Pathology. Hematological and clinical chemistry evaluations were performed on animals on Days -1, 7, and prior to necropsy on Day 15 or 21. In addition, clinical pathology evaluations were performed on blood samples collected from the female dog in the 320 mg/kg/day artelinic acid dose group (2607) prior to sacrifice on Day 4. Prior to the scheduled clinical pathology evaluations, animals were fasted overnight with access to water. Blood was drawn from a jugular vein into tubes containing EDTA (hematology evaluations) and into tubes with no anticoagulant (clinical chemistry evaluations). Blood samples were used for the following determinations:

Hematology:
Hemoglobin (HGB)
Hematocrit (HCT)
Total leukocyte count (WBC)
Erythrocyte count (RBC)
Platelet count (PLT)
Reticulocyte counts (RETIC)
Differential leukocyte count
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)

Clinical Chemistry:
Glucose (GLUC)
Creatinine (CREA)
Serum aspartate aminotransferase (AST)
Sodium (Na)
Potassium (K)
Chloride (Cl)
Blood urea nitrogen (BUN)
Serum alanine aminotransferase (ALT)
Alkaline phosphatase (ALP)
Gamma glutamyl transferase (GGT)
Lactate dehydrogenase (LDH)
Creatine kinase (CK)
Total protein (TP)
Globulin (calculated value)
Albumin
Albumin/globulin ratio (calculated value)
Blood urea nitrogen/creatinine ratio (BUN/CREA ratio; calculated value)

2.3.2.10 Blood Collections for Plasma Drug Level Determinations. On Days 1 and 14, a blood sample (~3-4 mL) was collected from each dog in dose Groups A, B, C, D, and E prior to dosing and at the following approximate times after dosing: 0.25, 0.5, 1, 2, 4, and 8 hours. In addition, on Days 7 and 15 or 21 (prior to necropsy), a blood sample was collected from each dog in Groups V, A, B, C, D, and E. The blood collection (~3-4 mL) on Day 7 was made immediately prior to administration of the daily dose of artelinic acid or arteether; the blood collection (~20 mL) on Day 15 or 21 was made at the time of sacrifice. Each blood sample was collected from the jugular vein into a tube containing heparin. Upon collection, each blood sample was centrifuged to obtain plasma. Plasma samples were stored at or below -70 °C prior to shipment, as directed by the Contracting Officer's Representative, for subsequent analysis. Artelinic acid concentrations were determined in plasma samples collected from dogs in Groups V, A, B, C, and D; these analyses were conducted in the laboratory of Dr. Emil Lin at the University of California at San Francisco. Arteether and DQHS concentrations were determined in plasma samples collected from dogs given arteether (Group E); these analyses were conducted by Dr. Qigui Li at WRAIR.

2.3.2.11 Necropsy and Histopathology. On Days 15 and 21, one dog of each sex in each dose group was sacrificed by an overdose of a barbiturate (pentobarbital; Butler Co.;

Columbus, OH). Liver samples (5-10 g) were removed from each animal, immediately frozen on dry ice, and then maintained at approximately –70 °C prior to subsequent shipment on dry ice to the Sponsor. All dogs then were perfused intracardially with a flush solution of 0.9% sodium chloride containing 1 mL of heparin (100 units/mL) and 1 mL of 1% sodium nitrate per liter. Immediately following the flush solution, each animal was perfused with an aldehyde fixative of 4% paraformaldehyde and 1% glutaraldehdye buffered with a phosphate buffer. The cranial, thoracic, abdominal, and pelvic cavities were opened, and the tissues/organs within each cavity were inspected. The tissues listed below were removed for standard histopathological evaluation, and the central nervous system was removed for neurotoxicological evaluation.

Adrenals
Aorta (aortic arch)
Bone, distal femoral head
Bone marrow (section from sternum and costochondral junction with rib)
Cecum
Colon
Duodenum
Esophagus
Eyes (including optic nerve and optic disk)
Gallbladder
Gross lesions
Heart (both atria, both ventricles, and intraventricular septum)
Ileum
Jejunum
Kidneys (2)
Liver (right medial lobe with section of gall bladder and left lateral lobe)
Lungs (left apical and left diaphragmatic lobes)
Lymph nodes (bronchial, mandibular, and mesenteric)
Mammary gland (left inguinal, when present)
Ovaries (2)
Pancreas
Pituitary
Prostate
Salivary gland
Sciatic nerve
Skeletal muscle
Skin [nonfrictional site (dorsal thorax); frictional site (elbow)]
Spinal cord (sections from the cervical and lumbar regions)
Spleen
Stomach (cardiac, pyloric, and fundic areas)
Testes (epididymides attached)
Thyroid and parathyroid (2)
Thymus
Tongue (including dorsal and lateral areas)
Tonsil (palatine)
Trachea
Ureter
Urinary bladder (fundus)

The brains and spinal cord sections were removed at necropsy and stored in fresh perfusion fixative. Eyes were saved in Davidson's solution. All other tissues (including the animal identification) were saved in 10% neutral buffered formalin.

Individual brains were blocked transversely, dehydrated in ascending grades of ethanol, and embedded in paraffin. The entire hindbrain was sectioned, mounted, and every 20th to 23rd section was stained with H & E or Luxol Fast Blue with cresyl violet.

All tissues (except brain and spinal cord) from all dogs were trimmed, embedded in paraffin blocks, and sectioned (approximately 5-µm sections). Representative tissue sections were mounted on glass slides, stained with hematoxylin and eosin (H & E), and coverslipped for histopathologic examination. All slides were submitted to a veterinary pathologist for evaluation and diagnosis.

Individual slides were evaluated by a pathologist at Southern Research, and lesions were graded on a five point scale with 0 as normal and 4 as the most severe. For the brain slides, the delineation of the scale used was as follows:

- 0 = normal
- 1 = trace; one or two degenerative or necrotic neurons present in one brain nucleus.
- 2 = mild; three or more degenerative or necrotic cells present in one brain nucleus or one or two degenerative cells in two nuclei of the brain
- 3 = moderate; more than half of the neurons in a nucleus showing degenerative or necrotic changes or 3 or more degenerative neurons in 3 or more brain nuclei
- 4 = severe; most or all of the neurons in a nucleus necrotic with a cellular response to the necrosis or many degenerative and necrotic neurons in numerous brain nuclei with evidence of cellular response to the necrosis.

2.3.2.12 Data Analyses: Plasma concentration data for artelinic acid were subjected to noncompartmental and compartmental pharmacokinetic analysis at Southern Research using WinNonlin (Version 1.1, Scientific Consulting, Inc.; Apex, NC). Plasma concentration data for arteether were subjected to noncompartmental analysis using WinNonlin.

In that this was a preliminary evaluation and only a limited number of animals were included in each dose group, no statistical analyses of the data were performed.

2.3.3 Results

2.3.3.1 Dose Formulation Analyses

The results of the analyses of the dose formulations of artelinic acid for chemical concentration are presented in Table 26. Two separate batches of each dose formulation, containing either 2, 4, 8, or 32 mg/mL of artelinic acid, were prepared during the study, one week apart. During the initial analysis of each formulation (conducted on either 10/4-5/99 or 10/11/99), the concentration of artelinic acid in each formulation was determined to be between 89 and 99% of the theoretical concentration, with two exceptions. These exceptions were for the 2 and 32 mg/mL artelinic acid formulations that were prepared for administration during the second week of dosing (date of analysis: 10/11/99); the measured concentration of artelinic acid in these two formulations was 78.0 and 81.1%, respectively, of the theoretical concentration. Six additional samples were subsequently obtained from each of these two formulations and the samples were analyzed (10/13/99) for artelinic acid concentration. Upon re-analysis, the mean concentration of artelinic acid in the samples removed from the 2 mg/mL formulation was determined to be 80.6% of theory (1.61 mg/mL) and that in the samples removed from the 32 mg/mL formulation was 96.7% of theory (30.9 mg/mL). Thus, the mean concentration of artelinic acid in the 2 mg/mL formulation, determined from the results of the analyses conducted on two separate dates, was 79.3% of the theoretical concentration; the mean concentration of artelinic acid in the 32 mg/mL formulation was 88.9% of the theoretical concentration.

The dose formulations of artelinic acid that analyzed at 89% or higher of the theoretical concentration were considered to be within specifications. Thus, all dose formulations used in the study met this criterion with the exception of the 2 mg/mL artelinic acid formulation prepared for use during the second week of dosing (Days 9-14). In that this dose formulation was given to animals in the lowest dose group, the Study Director did not consider this to have had an impact on the outcome of the study.

The measured concentration of arteether in the one arteether formulation prepared and administered during the study was 95.6 mg/mL, which was 95.6% of the theoretical concentration. The results of the analyses of samples removed from the top, middle, and bottom of the formulation indicated the formulation was homogenous.

2.3.3.2 Mortality

On Day 4, one female dog in the 320 mg/kg/day artelinic acid dose group displayed convulsions, emesis, prostration, and salivation shortly after administration of the daily dose of artelinic acid. It appeared likely that the animal had aspirated a portion of the dosing formulation; this lead to the ultimate sacrifice of the animal within a few hours after dosing.

One female dog in the 20 mg/kg/day arteether dose group displayed hypoactivity on Days 11-15, and then became extremely hyperexcited. The dog was euthanized at that point because of the threat of serious bodily injury to herself and/or the technical staff.

All other dogs in the study survived until their scheduled necropsy.

2.3.3.3 Clinical Signs

With the exception of a sore/ulcer that was noted on the right forefoot of 1/2 female dogs in the vehicle control group (Day 9), no adverse clinical signs were observed for the male or female dogs in the vehicle control group (0 mg/kg/day). Sporadic diarrhea was noted for one or more male or female dogs in each of the artelinic acid dose groups (20, 40, 80, or 320 mg/kg/day) and the arteether dose group (20 mg/kg/day); the etiology of the individual episodes of diarrhea was not known. No other adverse clinical signs were observed in dogs given either 20, 40, or 80 mg/kg/day of artelinic acid.

For dogs given 320 mg/kg/day of artelinic acid, hypoactivity, starting on Day 11 of the 14-day dosing interval, was noted for 1/2 female dogs in this dose group. As noted above, convulsions, emesis, prostration, and salivation were observed shortly after dosing on Day 4 for the other female dog in the 320 mg/kg/day artelinic acid dose group; these clinical signs were possibly related to aspiration of the dose formulation.

For dogs given 20 mg/kg/day of arteether, 1/2 female dogs in this dose group displayed ataxia (Days 10-12), hypoactivity (Days 10-14), edema surrounding the right eye (Days 10-14), prostration, excessive salivation, and tremors (Day 15). Hypoactivity (Days 11, 12, and 15) and subsequent hyperactivity (Day 15) were observed for the other female dog in this dose group; the extreme hyperactivity exhibited by this dog resulted in its euthanasia on Day 15, as described above.

2.3.3.4 Body Weights

Body weights for individual dogs are presented in Table 27. Body weight loss (greater than 10%) was noted between Day 1 and the day of sacrifice for 1/2 male dogs and one female dog in the 320 mg/kg/day artelinic acid dose group, and for 1/2 male and 2/2 female dogs in the 20 mg/kg/day arteether dose group.

2.3.3.5 Hematology

Decreased RBC (70% to 80% of baseline values), HGB, HCT, and/or reticulocyte counts ($\leq$ 33% of baseline values) which were observed in male and female dogs in the 20, 40, 80, and 320 mg/kg/day artelinic acid dose groups and 20 mg/kg/day arteether dose group were considered potentially drug-related effects. The decreases in RBC, HGB, and HCT values were considered to have minimal toxicologic relevance because the decreases represented a <10% overall decrease over that associated with blood collection alone (as observed in dogs in the vehicle control group). Increased reticulocyte counts (>2.0 fold baseline values) were observed on Day 21 in male dogs in the 40, 80, and 320 mg/kg/day artelinic acid dose groups and 20 mg/kg/day arteether dose group, a finding that was also considered potentially drug related. Biologically relevant changes were defined as changes that were greater than those consistent with expected intraindividual and assay variability.

Decreased RBC (70% to 80% of baseline values), HGB, or HCT that were observed on Day 7 or 15 in 2/4, 4/4, 4/4, 2/3, and 2/4 dogs in the 20, 40, 80, and 320 mg/kg/day artelinic

acid dose groups and 20 mg/kg/day arteether dose group were considered potentially drug-related effects. Decreased reticulocyte values (≤33% of baseline values) that were observed on Day 7 or Day 15 in 2/4, 4/4, 3/4, 3/3, and 4/4 dogs in the same dose groups were also considered potentially drug-related effects. Increased reticulocyte counts (>2.0 fold baseline values) were observed on Day 21 (7 days post dosing) in male dogs in the 40, 80, and 320 mg/kg/day artelinic acid dose groups and 20 mg/kg/day arteether dose group; the increased reticulocyte counts were consistent with a bone marrow rebound effect for erythropoiesis. While RBC, HGB, and HCT values were also observed to be variably decreased in dogs in the vehicle control group, a slightly greater magnitude of decrease was evident in individual dogs in the artelinic acid and arteether dose groups. Minimal RBC counts observed for male and female dogs in the vehicle control group ranged from 81% to 89% of baseline counts for male dogs and 82% to 96% of baseline counts for female dogs. In comparison, minimal RBC counts observed for dogs in the artelinic acid and arteether dose groups ranged from 70% to 85% for male dogs and 77% to 90% for female dogs (excluding DF2607 that was euthanized on Day 4). No biologically relevant (changes greater than those consistent with expected intraindividual and assay variability) decreases (≤33% of baseline values) in reticulocyte counts or post-dosing increases in reticulocyte counts (>2.0 fold baseline values) were observed in dogs in the vehicle control group. Comparable blood volumes were withdrawn from dogs in both the vehicle and artelinic acid and arteether dose groups.

Greater variability in WBC, neutrophil, and monocyte counts (occasional WBC and neutrophil counts 150 to 208% of baseline counts; occasional monocyte counts 2.0 to 7.3 fold greater than baseline counts) was observed in dogs in the artelinic acid dose groups than in dogs in the vehicle control group. Due to the lack of a definitive dose-related effect, the variability observed in WBC, neutrophil, and monocyte counts was probably an incidental finding and not drug related.

Drug-related decreased leukocyte and neutrophil counts were observed for male and female dogs and decreased lymphocyte counts were observed for female dogs in the 20 mg/kg/day arteether dose group. Minimal leukocyte counts observed in 1/2 male and 2/2 female dogs in the 20 mg/kg/day arteether dose group ranged from 30% to 53% of baseline counts. Minimal neutrophilic counts observed in 1/2 male and 2/2 female dogs in the 20 mg/kg/day arteether dose group ranged from 9% to 39% of baseline counts. A decreased lymphocyte count was only observed in 1/2 female dogs in the 20 mg/kg/day arteether dose group with a lymphocyte count 58% of the baseline count. The decreases in WBC, neutrophil, and lymphocyte counts observed were considered drug-related effects.

2.3.3.6 Clinical Chemistry

Decreased albumin and A/G ratios and/or increased globulin concentrations, observed in female dogs in the 80 (1/2) and 320 mg/kg/day artelinic acid dose groups and in male and female dogs in the 20 mg/kg/day arteether dose group were considered the predominant drug-related findings. Other findings that were considered to be potentially drug related in dogs in the 20 mg/kg/day arteether dose group were moderate increases in ALP, AST, LDH, and/or CK activity.

Biologically relevant decreases in albumin concentration were observed in 1/2 female dogs in both the 80 and 320 mg/kg/day artelinic acid dose groups with minimum albumin concentrations 88% and 79%, respectively, of the corresponding baseline values. Minimum albumin concentrations which were also observed for male and female dogs in the 20 mg/kg/day arteether dose group ranged from 65% to 85% of the corresponding baseline values. Biologically relevant increased globulin concentrations were observed in 2/2 female dogs in the 320 mg/kg/day artelinic acid dose group and in 2/2 male dogs and 1/2 female dogs in the 20 mg/kg/day arteether dose group with maximum values ranging from 118% to 200% of baseline values. Decreases in the A/G ratio were associated with the increased globulin and decreased albumin concentrations. The etiology for the decreased albumin and increased globulin concentrations was not determined by this study. Diarrhea, a potential cause for loss of protein, was observed in female dogs in the 320 mg/kg/day artelinic acid dose group and the 20 mg/kg/day arteether dose group; therefore, gastrointestinal loss was one possible source of albumin loss. Weight loss with decreased intake of protein also potentially contributed to the decreased albumin concentrations observed. The increased globulin concentration suggested that inflammation was present, so albumin may also have been decreased in response to a chronic inflammatory stimulus.

Moderate increases in LDH activity (moderate increases: ≥4.0-fold to <7.0-fold greater than baseline values) were observed in 1/2 male dogs in the 80 mg/kg/day artelinic acid dose group, 1/2 male and female dogs in the 320 mg/kg/day artelinic acid dose groups and 1/2 male and female dogs in the 20 mg/kg/day arteether dose groups. A moderate increase in CK activity (≥4.0-fold to <8.0-fold greater than baseline values) was observed in 1/2 female dogs in the 20 mg/kg/day arteether dose group on Day 15. Increases in ALP activity were also observed rarely in 1/2 male and 2/2 female dogs in the 20 mg/kg/day arteether dose group with maximum values observed that ranged from 2.5-fold to 4.7-fold greater than baseline values. Increased ALT activity (maximum value 5.8-fold greater than baseline value) was observed in 1/2 female dogs in the 80 mg/kg/day artelinic acid dose group. The increased ALT activity observed in this dog on Day 21 was possibly associated with the mottled liver observed at necropsy on the same day; however, due to the lack of observations of elevated ALT activities for dogs in the 320 mg/kg/day dose group and only an incidental finding of mild diffuse hepatocellular necrosis in 1/4 dogs in the 320 mg/kg/day dose group, this change was probably not drug related. Increased AST activity (maximum value 5.7-fold greater than baseline value) was observed in 1/2 female dogs in the 20 mg/kg/day arteether dose group. Increases in ALP, CK, LDH, and AST values observed in dogs in the 20 mg/kg/day arteether dose group were considered to be potentially drug related due to the multiple enzymes affected. These changes were probably secondary to intestinal toxicity; the increased CK values observed in one female dog in the 20 mg/kg/day arteether dose group was also potentially associated with the clinical signs of tremors and prostration observed for this dog on Day 15. The sporadic increases in CK, ALT, ALP, AST, and LDH activity observed in dogs in the artelinic acid dose groups did not appear to be dose related; therefore, the effects were not considered drug related. Mild increases in LDH activity (<4.0-fold greater than baseline values and >100 U/L) and mild increases (and decreases if baseline values high) in CK activity (<4.0 fold) were observed sporadically in dogs in the vehicle control (females only) and artelinic acid and arteether dose groups; however, these were not considered to be drug related.

Other findings that were abnormal, but not determined to be drug related included decreases in potassium concentrations, and decreases and increases in glucose concentration. Biologically relevant decreases in potassium concentrations (values 70% to 88% of baseline values) occurred in 1/2 male dogs in the 20 mg/kg/day artelinic acid dose group, 1/2 female dogs in the 40, 80, and 320 mg/kg/day artelinic acid dose groups, and 1/2 male dogs in the 20 mg/kg/day arteether dose group. The sporadic decreases in potassium concentration that occurred were not consistently related to findings of diarrhea; therefore, these changes were not considered to be drug related. An increased glucose (value 311% of baseline value) was observed in 1/2 female dogs in the 320 mg/kg/day artelinic acid dose group that was sacrificed on Day 4; thus, this value was consistent with stress hyperglycemia. An extremely low glucose value (6 mg/dL) observed in 1/2 female dogs in the 20 mg/kg/day arteether dose group was not considered to be consistent with a biological value from a living dog and was interpreted as consistent with preanalysis depletion of glucose (sample was hemolyzed) or other random analytical error. The dog with the low glucose value was not observed to have any pancreatic histopathology changes consistent with diabetes.

One female dog in the 40 mg/kg/day artelinic acid dose group was observed to have increased potassium and total protein on Day 21 with respective values 126% and 111% of baseline values, both values that indicated dehydration; however, the cause for this transient dehydration was not determined.

2.3.3.7 Plasma Drug Levels

Plasma concentrations of artelinic acid on Days 1, 7, and 14 in male and female dogs in the vehicle control (0 mg/kg/day) and 20, 40, 80, or 320 mg/kg/day artelinic acid dose groups are presented in Table 28. Among dogs in the individual dose groups, no sex-related differences in plasma concentrations of artelinic acid were apparent at any time during the study.

On Day 1, peak plasma concentrations (Cmax) of artelinic acid were observed at 0.5-1 hour after dosing among dogs in each dose group given artelinic acid; artelinic acid was not detected in plasma collected from dogs in the vehicle control group. At 0.5 –1 hour after dosing on Day 1, plasma concentrations of artelinic acid were 9440-13900, 19600-26700, 28900-35300, and 53100-160000 ng/mL for dogs in the 20, 40, 80, and 320 mg/kg/day dose groups, respectively.

For plasma samples collected prior to dosing on Day 7, low levels of artelinic acid were detected in plasma collected from the three surviving dogs in the 320 mg/kg/day dose group. For plasma samples collected prior to dosing on Day 14, low levels of artelinic acid were detected in plasma collected from one dog in the 20 mg/kg/day dose group and from one dog in the 320 mg/kg/day dose group.

On Day 14, plasma concentrations of artelinic acid in individual dogs were not discernibly different from those observed on Day 1.

Plasma concentrations of arteether and its metabolite, DQHS, on Days 1, 7, and 14 in dogs given an im dose of 20 mg/kg/day of arteether are presented in Appendix B. No sex-related

differences in the plasma concentrations of arteether and DQHS were apparent. On Day 1, peak plasma concentrations of arteether, ranging from 75.13 to 83.36 ng/mL, were observed between 1 and 2 hours after dosing. With repeated administration plasma concentrations of arteether increased. Prior to dosing on Day 7 (approximately 24 hours after the previous dose), plasma concentrations of arteether ranged from 126.29 to 131.05 ng/mL; slightly higher concentrations were observed on Day 14 prior to dosing. After dosing on Day 14, peak plasma concentrations of arteether were approximately 3- to 4-fold higher than those observed at the corresponding time on Day 1 and ranged from 247.52 to 300.02 ng/mL. On all days of analysis, plasma concentrations of DQHS were lower than those observed for arteether; however, plasma concentrations of DQHS were higher on Day 14 than on Day 1.

2.3.3.8 Pharmacokinetic Analyses

Pharmacokinetic parameters estimated from noncompartmental analysis of plasma concentration data for artelinic acid presented in Table 29. AUC values calculated from plasma concentrations of artelinic acid on Day 1 increased with increasing dose and the mean $AUC_{0-\infty}$ values were 13927, 33064, 52855, and 194955 ng•hr/mL for male and female dogs in the 20, 40, 80, and 320 mg/kg/day dose groups. Due to limited data points, only a single phase of elimination of artelinic acid was apparent for most dogs. On Day 1, the mean half-life of the initial phase of elimination of artelinic acid ranged from 0.38 to 0.59 hours among dogs in the 20, 40, and 80 mg/kg/day dose groups. The mean terminal elimination half-life of artelinic acid was 1.11 and 3.39 hours for dogs in the 80 and 320 mg/kg/day dose groups, respectively. Pharmacokinetic parameters calculated from plasma concentrations of artelinic acid on Day 14 were similar to those estimated from the plasma concentration data obtained on Day 1.

Plasma concentration data for arteether were also subjected to noncompartmental pharmacokinetic analysis. Pharmacokinetic parameters estimated for arteether are resented in Table 30. The mean $AUC_{0-\infty}$ value for male and female dogs given 20 mg/kg/day of arteether was 721 ng•hr/mL on Day 1 and 1683 ng•hr/mL on Day 14. The mean half-life of arteether in plasma was 7.65 hours on Day 1 and 6.15 hours on Day 14, indicating that the elimination half-life of arteether was not changed by repeated administration of the compound.

2.3.3.9 Necropsy

A dark lung and mottled lung seen in high dose group animal 3F2607, which underwent moribund sacrifice on Day 4, were considered to be agonal changes associated with acute death. Dark kidney, mottled liver, dark lymph node, mottled adrenal gland, gelatinous pancreas, and spleen adhesion were seen randomly in dogs from various dose groups, and were considered to be incidental findings. Thin thymus (1/4) and gingival lesion (2/4) seen in positive control group dogs were considered drug-related changes. Enlarged lymph node and enlarged thyroid gland seen in a single positive control group dog also were considered to be incidental findings.

2.3.3.10 Histopathology

Histopathological findings observed during the evaluation of brains are presented in Table 31.

There were no lesions present in sections of the brain from dogs treated with either 20, 40, 80, or 320 mg/kg/day of artelinic acid or among dogs in the vehicle control group.

Neuron degeneration occurred in widely scattered nuclei of the hind brain in the male and female dogs treated with 20 mg/kg/day of arteether. The severity of the degeneration was determined on a four point scale depending on the number of neurons involved. Severities of +2 to +4 were observed in the four dogs treated with arteether. The neuron degeneration was characterized by loss of Nissl staining, swelling of the cytoplasm, and margination of the nucleus. These cells had a red/brown cytoplasm on haemotoxylin and eosin stained sections. More advanced degeneration was seen in a few neurons, with clumping of eosinophilic debris in the swollen cytoplasm.

Histopathologic evaluations were also conducted on the nonbrain tissues obtained from male and female dogs in the 0 and 320 mg/kg/day artelinic acid dose groups and in the 20 mg/kg/day arteether dose group. These findings are presented in Table 32. No lesions considered to be directly related to administration of artelinic acid were observed.

In the three dogs in the 320 mg/kg/day artelinic acid dose group from the Day 15 or Day 21 scheduled sacrifices, thymic atrophy of moderate severity was seen in the male and female dogs sacrificed on Day 21. Thymus was not available for evaluation in the male dog sacrificed on Day 15. Mild testicular hypocellularity and cellular atypia were seen unilaterally in the male dog sacrificed on Day 15. Thymic and testicular changes similar to those seen in these dogs are occasionally observed in untreated beagles, and they were considered to be incidental in this study. Other lesions observed in the three artelinic acid high dose group dogs from the scheduled sacrifices included pituitary gland cyst (1/3) and mild skeletal muscle hypercellularity (1/3). These changes were also considered to be incidental.

The female dog in the 320 mg/kg/day artelinic acid dose group, which underwent moribund sacrifice on Day 4, had lung hemorrhage, lung edema, adrenal gland congestion, and bronchial lymph node hemorrhage of mild to marked severity, as well as diffuse hepatocellular necrosis of minimal severity. These were considered to be agonal lesions typical of those associated with acute death, the cause of which may have been related to aspiration of the dose formulation. This animal also had a pituitary gland cyst, which was considered an incidental finding.

In the absence of mortality in the remaining artelinic acid-treated dogs and of test article-related lesions in the three dogs in the 320 mg/kg/day dose group from the scheduled sacrifices, evaluation of tissues from dogs given lower doses of artelinic acid was not deemed necessary.

Lesions considered to be directly or indirectly test article-related in the four dogs (two male and two female) given arteether included: bone marrow hyperplasia (2/4), spleen hematopoietic cell proliferation (3/4), spleen lymphoid depletion (2/4), lymph node lymphoid depletion (2/4), tonsil lymphoid depletion (1/4), liver hematopoietic cell proliferation (3/4), skeletal muscle inflammation (1/4), and oral mucosa erosion or ulcer (2/2). Marked lymphoid depletion in the thymus of animal 6F2610 may have been test article related. The remaining lesions seen in the arteether dogs were considered to be incidental or of uncertain cause.

2.4 Task Order SR00-1: Study of Injectable Artelinic Acid in Dogs

2.4.1 Objective

When artelinic acid (AL) in lysine at a concentration of 32 mg/mL was administered to rats via a tail vein at a dose of 16 mg/kg daily for 3 days, venulitis, as evidenced by edema and color change, was seen. It was hypothesized that the reason for AL venotoxocity was the small size of the vein used for AL injection. If so, AL should not be toxic to dog peripheral vein and would, therefore, be appropriate for IV treatment of humans. The clinical dose of artesunate, and perhaps artelinate, is 2 mg/kg over 5-10 minutes using a 10 mg/mL solution. In the study described herein, the dose/concentration or doses/concentration of artelinic acid were 3 to 6-fold higher than those for artesunate.

The objective of this study was to assess the occurrence of any clinical and/or observable effects on and around the injection site following IV injection of single, daily doses of an artelinic acid/lysine formulation, equal to or greater than the anticipated clinical doses, to dogs for 7 consecutive days.

2.4.2 Materials and Methods

2.4.2.1 Test System. The male and female dogs used in this study were obtained from Marshall Farms (North Rose, NY). On the day of dosing, the dogs were 8-9 months of age and weighed between 7 and 9 kg.

Upon arrival, each dog was placed in quarantine and given a physical examination. There were no significant findings indicative of poor health, and the laboratory veterinarian released these animals for study. Housing, feed, and water procedures remained the same during the quarantine and study periods.

The dogs were exposed to their daily ration of commercial, dry Certified Canine Diet #5007 (PMI Feeds, Inc., St. Louis, MO) for a total period of approximately 2 hours per day. The quantity of the daily ration was sufficient to meet nutritional requirements. The water source was the public supply (Birmingham public water supply) and was given *ad libitum.* The dogs were individually housed in stainless steel cages in a room that was maintained at a temperature of 69-75°F and a relative humidity of 35-65%. Room lights were controlled by an automatic timer set to provide 12 hours of light (0600 to 1800 hours, CST) and 12 hours of dark per day. Cage size and animal care conformed to the guidelines of the *Guide for the Care and Use of Laboratory Animals*, 7th edition[6] and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198) and to the applicable Standard Operating Procedures (SOPs) of Southern Research.

Each dog was identified by an ear tattoo or letter combination. Dogs are an accepted species to support pharmacological and toxicological evaluations of drugs used or intended for use in humans.

2.4.2.2 Test Articles. One shipment containing 30 grams of artelinic acid/lysine salt (AL/lysine) was supplied by Walter Reed Army Institute of Research (WRAIR), Washington, DC. The test article was stored frozen until used and is considered stable as a powder when stored under these conditions.

2.4.2.3 Vehicle and Vehicle Control. The reagents used for the preparation of AL/lysine dose formulations are listed below.

- Purified L-lysine (supplied by WRAIR; expiration date not supplied; stored frozen at approximately -20°C)
- L-lysine (Aldrich Chemical Company; Milwaukee, WI; Lot 08325JI; Southern Research assigned expiration date January 31, 2003; stored at room temperature)
- Sterile water (Phoenix Pharmaceutical, Inc.; St. Joseph, MO; Lot 101002F; expiration date January 2004; stored at room temperature)
- Sterile saline (Phoenix Pharmaceutical, Inc.; St. Joseph, MO; Lot 0081050; expiration date August 2003; stored at room temperature)
- Hydrochloric acid (HCl; EM Sciences; Gibbstown, NJ; Lot 32232; Southern Research assigned expiration date January 31, 2003; stored at room temperature)

2.4.2.4 Dose Formulation Preparation. The vehicle control formulations were prepared by dissolving L-lysine in sterile saline to yield a final concentration of 11.5 mg/mL of L-lysine. For preparation, the L-lysine was weighed into a volumetric flask and diluted to the desired volume with sterile saline. The contents were stirred until well mixed and then the solution was adjusted to pH 9 with hydrochloric acid. The vehicle control formulations were then filter sterilized using a 0.2 μm cellulose acetate filter and used for dosing within 7 days after preparation.

AL/lysine dose formulations were prepared by making a stock solution containing 30 mg/mL of AL; the artelinic acid content of the preformulated AL/lysine salt was equivalent to 1 gram of artelinic acid per 1.35 gram of salt. The required amount of AL/lysine salt was weighed into a volumetric flask and QS to the mark with a solution containing 0.45% saline/0.1% purified L-lysine. The contents were stirred until well mixed and then filter sterilized using a 0.2 μm cellulose acetate filter. Next, a 10 mg/mL artelinic acid formulation was made by a 1 to 3 dilution of the 30 mg/mL stock formulation. This was prepared by diluting the required amount of 30 mg/mL formulation with a solution containing 0.9% saline/0.1% purified L-lysine and filter sterilizing using a 0.2 μm cellulose acetate filter.

Due to the fact that an insufficient quantity of purified L-lysine was received from WRAIR, a 1.15% solution of L-lysine, prepared from material purchased from Aldrich Chemical Company (97% purity), was used for some of the doses; specifically, this solution was administered to the dogs in the vehicle control group (Groups 1 and 2) on Days 5-7. For all other doses and for preparation of the artelinic acid formulations, the purified L-lysine was used.

All dose formulations were stored refrigerated in amber glass vials prior to use. Each formulation was used for dosing within 7 days after preparation and was considered stable for the period of use.

2.4.2.5 Dose Formulation Analysis. Each AL/lysine dose formulation was analyzed by HPLC at the time of preparation and at the end of the dosing period for chemical concentration.

2.4.2.6 Group Assignment. The male and female dogs were randomly assigned to five dose groups using a computer generated Artemis® randomization procedure. The dogs were weighed during Week -1, and these body weights were used to randomly assign the animals to one of 4 treatment groups or to a vehicle control group (1 dog/sex/dose for Groups 1 and 2; or 2 dogs/sex for Groups 3-5) as shown in the table below:

Group ID	Formulation	Approx. Dose[a] (mg/kg/day)	Dose Conc. (mg/mL)	Dose Volume (mL/day)	No. of Males	No. of Females
1	Vehicle Control	0	0	2	1	1
2	Vehicle Control	0	0	4	1	1
3	Artelinic Acid/Lysine	2	10	2	2	2
4	Artelinic Acid/Lysine	6	30	2	2	2
5	Artelinic Acid/Lysine	12	30	4	2	2

[a] Doses are based on a body weight of 10 kg.

2.4.2.7 Dose Procedure. Beginning on Day 1, each dog was given an iv dose of either the test or control article. The dose volumes were as indicated in the above table. All doses were administered into a peripheral leg vein and delivered over an approximate 10-minute interval, using an infusion pump. For each dog, the dose was administered in the same vein each day, where possible.

2.4.2.8 Body Weights. Body weights for all animals were obtained during Week –1 and on Days 1-7 (prior to dosing).

2.4.2.9 Clinical Observations. On Days 1-14, each dog was observed at least twice daily for signs of clinical toxicity. Each evaluation included an assessment of posture, activity, level of arousal, and breathing. The site of injection was closely examined for irritation/swelling or other adverse reactions.

2.4.3 Results

2.4.3.1 Clinical Observations

No drug-related effects on body weight were observed for any dog on study (Figure 10). In addition, no adverse reactions at the injection site or clinical signs of toxicity were observed for any dogs given the vehicle control formulation (either 2 mL or 4 mL; Groups 1 and 2), or for dogs given 2 mL of either the 10 or 30 mg/mL AL/lysine formulation (Groups 3 and 4), or for the male dogs given 4 mL of the 30 mg/mL AL/lysine formulation (Group 5). The only adverse reactions noted were for the two female dogs given 4 mL of 30 mg/mL AL/lysine (Group 5). These two dogs displayed swelling in the leg, near the injection site, beginning on Day 5 or 6 of dosing and continuing through Day 10 or 11; no necrosis, discoloration, or other adverse signs were evident. Note: For one of these female dogs in Group 5, difficulty was encountered

inserting the catheter for infusion into the front leg vein on each day of dosing; for this reason, not all doses given to this dog were delivered into the same leg vein.

2.4.3.2 Dose Formulations

At the time of preparation the dose formulations of AL/lysine and L-lysine were clear and colorless. The experimentally determined concentration of AL in the two AL/lysine dose formulations prior to dosing was 10.1 and 30.8 mg/mL. Thus, the concentration of AL in each formulation was within 3% of the theoretical concentration.

The dose formulations were carefully evaluated after the end of the 7-day dosing period. This evaluation included a visual inspection for color/precipitation, pH determination, and HPLC analysis for AL concentration.

Upon visual examination 8 days after preparation, the vehicle control formulation and the 10 and 30 mg/mL AL/lysine formulations appeared clear; no particulate matter was evident in any of these three formulations. The vehicle control formulation was colorless; however, the two formulations of AL/lysine were pink in appearance. The pink color was darker in the 30 mg/mL formulation than in the 10 mg/mL formulation. The pinkish appearance of the AL/lysine formulations was not evident when the formulations were drawn into a 5 cc syringe, as was used for dose administration. Due to the fact that the dose formulations were maintained in amber colored bottles, it was not known when the color change occurred. At the end of the dosing period, the pH of the 10 mg/mL AL/lysine formulation was 8.4 and that of the 30 mg/mL formulation was 8.2. [Note: pH determinations were not made on the formulations at the time of preparation].

The results of the post-dose HPLC analyses indicated the concentration of AL in the 10 and 30 mg/mL AL/lysine formulations did not change during the period of use. After the end of the dosing period, the AL content of the two formulations was determined to be 10.0 and 30.1 mg/mL.

2.5 Task Order SR01-1: Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

2.5.1 Objective

Artemisinin derivatives differ from quinine/quinidine, the traditional treatments of severe malaria, in that they kill parasites much more rapidly. In that most mortality from severe malaria occurs in the first 24-48 hours, this class of compounds should theoretically reduce the mortality rate. The results of several large well-controlled studies, however, do not support this. It has been proposed that the lack of improvement may be due to slow and/or poor absorption following intramuscular administration of these oil soluble derivatives. Artesunate suppositories are being developed for initiation of treatment of critically ill patients where medical facilities are not available; however, bioavailability and variability of absorption are also issues with suppositories.

An intravenous (iv) formulation ensures 100% bioavailability with immediate peak concentrations. Intravenous artesunate (AS) is currently available in China; however, only artelinate (AL) appears to have sufficient stability in solution to allow the drug to be available in a "ready-to-use" injection vial. The currently marketed AS formulation is a freeze-dried preparation, which requires mixing with a sodium bicarbonate solution at the bedside and then dilution with dextrose in water.[10] An artelinate/lysine formulation has recently been developed and is being evaluated for suitability as an iv injectable solution.

The objective of this study was to conduct a dose range-finding evaluation of the pharmacodynamic effects of AL and AS, as assessed by signs of clinical and pathological toxicity, following iv administration for 7 consecutive days. During Phase 1 of this study, an estimate of the LD_{50} of a single iv dose of AL or AS was obtained. This information was used to determine doses of AL or AS to be given during a subsequent 7 day range finding toxicity study that was conducted in two phases (Phases 2 and 3). The results of all phases of the study are described herein.

2.5.2 Materials and Methods

2.5.2.1 Test System. The rats used for this study were received in three separate shipments. The 24 male and 24 female rats designated for use in the LD_{50} phase of this study were selected from among 29 male and 28 female Sprague-Dawley [Crl:CD(SD)IGS BR] rats received from Charles River Laboratories, Inc. (Raleigh, NC; Area R05), on September 18, 2001. Sprague-Dawley [Crl:CD(SD)IGS BR] rats (39 males and 40 females) were received for Phase 2 of the range-finding study on October 16, 2001, and additional Sprague-Dawley [Crl:CD(SD)IGS BR] rats (17 males and 16 females) were received for Phase 3 of the range-finding study on April 30, 2002. All rats in each shipment were 6-8 weeks old when they arrived at Southern Research Institute (Southern Research). Individual animal identification was by ear punch. The Sprague-Dawley rat is an accepted species to support clinical studies of drugs or chemicals used or intended for use in humans.

Upon arrival, the rats were placed in quarantine. The animals were examined for general health within 3 days of receipt. There were no significant findings indicative of poor health, and the animals were released for study. Each rat was allowed access to feed (Certified Rodent Diet #5002; PMI Feeds, Inc.; St. Louis, MO) and tap water (Birmingham public water supply) *ad libitum* during the quarantine and study periods. No known contaminants were present in the food or water that could interfere with or affect the outcome of the study. The rats were individually housed in solid-bottom, polycarbonate, shoebox cages on stainless steel racks during the quarantine and study periods in an animal room that was maintained at a temperature of 71.2-75.7 °F and a relative humidity of 49.0-80.0%; excursions outside the desired relative humidity range (50±20%) that occurred were brief and did not affect the outcome of the study. Hardwood chip bedding (P.J. Murphy Forest Products, Inc.; Montville, NJ) was used in the cages for excrement absorption. Room lights were controlled by an automatic timer set to provide approximately 12 hours of light (approximately 0600 to 1800 hours, CST) and 12 hours of dark per day. Cage size and animal care conformed to the Guidelines for the Care and Use of

Laboratory Animals, 7th edition[6] and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198).

2.5.2.2 Test Articles. One bottle containing 30 grams of artelinic acid/lysine salt (AL/lysine; WR 255663; Bottle No. BP21847; manufacturer's code 2117-308; Southern Research Lot D78/L-6; expiration date not supplied) was supplied by Walter Reed Army Institute of Research (WRAIR; Washington, DC) and received at Southern Research on April 18, 2001. The bulk quantity of AL/lysine was stored frozen (-20 °C) from the time of receipt until the time of use (9/25/01 through 5/8/02) and was assumed to be stable when so stored.

One bottle containing 200 grams of artesunic acid (artesunate; AS; WR 256283; Bottle No. BP18288; Lot No. 1 03; Southern Research Lot E38/L-2; expiration date not supplied) was supplied by WRAIR and received at Southern Research on September 18, 2001. The bulk quantity of AS was stored frozen (-20 °C) from the time of receipt until the time of use (9/26/01 through 5/15/02) and was assumed to be stable when so stored.

One bottle containing 3 grams of arteether (AE; WR 255131; Bottle No. BP23788; manufacturer's code FJ18-97-3; expiration date not supplied) was supplied by WRAIR and received at Southern Research on August 29, 2001. The bulk quantity of AE was stored refrigerated from the time of receipt until the time of use (10/23/01) and was assumed to be stable when so stored.

2.5.2.3 Vehicles. The reagents used for preparation of the dose formulations and vehicle control formulations of AL/lysine, AS, and AE were as follows:

- Purified L-lysine (WRAIR; Washington, DC; Bottle Number BP23761; stored refrigerated/dark).
- L-lysine monohydrochloride (WRAIR; Washington, DC; Bottle Number BP23770; stored refrigerated/dark).
- L-lysine (Aldrich; Milwaukee, WI; Lot KI08325JI, Southern Research assigned expiration date January 31, 2003; stored at room temperature).
- Sesame oil (Sigma; St. Louis, MO; Lot 90K0870, Southern Research assigned expiration date January 31, 2003; stored at room temperature).
- Sodium bicarbonate (Fisher; Fair Lawn, NJ; Lot 016178, Southern Research assigned expiration date January 31, 2003; stored at room temperature).
- Sterile saline (Phoenix Pharmaceutical, Inc.; St. Joseph, MO; Lot 102049F, expiration date February 2004; Lot 104113F, expiration date April 2004; Lot 104114F, expiration date April 2004; Lot 112385F, expiration date December 2004; stored at room temperature).
- Sterile water (Phoenix Pharmaceutical, Inc.; St. Joseph, MO; Lot 104129F, expiration date April 2004; Lot 109276F, expiration date September 2004; Lot 112384F, expiration date December 2004; stored at room temperature).

2.5.2.4 Dose Formulation Preparation.

AL/lysine. The AL/lysine salt prepared by WRAIR was used for preparation of all dose formulations of AL; the preformulated AL/lysine salt contained 1 gram of artelinic acid per 1.35 g of salt.

Dose formulations of AL/lysine containing 4, 8, 16, 32, or 64 mg/mL of AL were prepared for use during the LD_{50} phase of the study. For this, stock solutions containing either 16 or 64 mg/mL of AL were prepared by dissolving a calculated quantity of AL/lysine salt in 0.9%NaCl/0.3% purified L-lysine. The 64 mg/mL AL solution was diluted to 32 mg/mL using 1 volume of 0.9% NaCl/0.3% purified L-lysine. The 16 mg/mL AL/lysine solution was diluted to 4 or 8 mg/mL of AL using the appropriate volume of 0.9% saline/0.3% purified L-lysine.

Dose formulations containing 0.75, 1.5, 3, or 6 mg/mL of AL were prepared for use during the range-finding phase (Phases 2 and 3) of the study. These formulations were prepared by dissolving individual weighed quantities of AL/lysine salt in an appropriate volume of 0.9% NaCl/0.3% purified L-lysine.

The AL vehicle control dose formulations (0 mg/mL AL) contained 1.15% L-lysine monohydrochloride in 0.9% saline.

Each AL/lysine and corresponding vehicle control dose formulation was filtered through a 0.2-micron cellulose acetate filter immediately after preparation. The formulations were stored refrigerated during the period of use. AL has been shown to be chemically stable in AL/lysine solutions prepared in 0.9% NaCl/0.3% purified L-lysine for at least 8 days when maintained refrigerated.[11]

Artesunate. Dose formulations containing 10, 20, 40, or 80 mg/mL of AS were prepared for use during the LD_{50} phase of the study. For this, a stock formulation containing 133 mg/mL of AS was prepared in 5% sodium bicarbonate. This stock solution was diluted with sterile water to yield a formulation containing 80 mg/mL of AS and also diluted with 0.9% sterile saline to yield formulations containing 10, 20, or 40 mg/mL of AS.

Dose formulations containing 1.9, 3.75, 7.5, 15, and 30 mg/mL of AS were prepared for use during the range-finding phase (Phases 2 and 3). For this, stock formulations containing 120 mg/mL of AS were prepared in 5% sodium bicarbonate. The stock solutions were diluted, as appropriate, with 0.9% sterile saline, to yield formulations containing 1.9, 3.75, 7.5, 15, or 30 mg/mL of AS.

The AS vehicle control dose formulation (0 mg/mL AS) was prepared by diluting a solution of 5% aqueous sodium bicarbonate with 7 volumes of 0.9% sterile saline.

Each AS and corresponding vehicle control dose formulation was filtered through a 0.2-micron cellulose acetate filter immediately after preparation. The formulations were maintained at room temperature and used within 2 hours of preparation. Batty and

coworkers have shown that AS is stable for at least 4 hours in bicarbonate/saline solutions maintained at 23°C.[12]

Arteether. The dose formulation of arteether was prepared in sesame oil to contain AE at a concentration of 50 mg/mL. For preparation, a weighed quantity of AE was added to an appropriate volume of sesame oil and the mixture was sonicated and stirred until dissolution of the AE occurred. The AE dose formulation was stored at room temperature. Previous investigations have shown that AE is stable in sesame oil for at least 1 year when stored at room temperature.[8]

2.5.2.5 Dose Formulation/Homogeneity Analysis: Each AL/lysine dose formulation prepared during Phases 1, 2, or 3 was analyzed by HPLC at the time of preparation for chemical concentration and homogeneity. In addition, each AL/lysine formulation prepared during Phase 2 was analyzed for chemical concentration at the end of the dosing period to assess stability. The conditions of analysis were as described in Section 2.1.2.3.

Dose formulations of AS prepared for use during Phase 1 were analyzed by HPLC for chemical concentration and homogeneity immediately after preparation. During Phase 2, dose formulations of AS prepared for use on Days 1, 3, and 7 of the 7-day dosing period were analyzed for chemical concentration and homogeneity. During Phase 3, dose formulations of AS prepared on Days 1 and 7 were analyzed. The conditions of analysis were as follows:

Column:	Bondapak C18, 10 µm, 300 mm x 3.9 mm (Phenomenex, Torrance, CA)
Mobile Phase:	65% Methanol:35% 0.1 M aqueous ammonium acetate
Elution:	Isocratic
Temperature:	Ambient
Flow rate:	1.5 mL/min
Detection:	UV absorbance at 210 nm

The dose formulation of AE prepared during Phase 2 was analyzed by HPLC at the time of preparation for chemical concentration and homogeneity. The conditions of analysis were as described in Section 2.2.2.5.

2.5.2.6 Group Assignment: For each study phase, the male and female rats were randomly assigned to dose groups using an Artemis® (Liverpool, Great Britain) computer-generated randomization procedure. For each randomization, the rats were weighed during Week -1, and the mean body weight by sex was used to determine the acceptable weight range. Rats closest to the mean weight by sex were selected for study. Dose group assignments by phase are presented in the following tables.

LD_{50} (Phase 1)

Group ID	Formulation	Nominal Dose		Number of Rats	
		Level (mg/kg)	Concentration (mg/mL)	Males	Females
1	AL Vehicle Control	0	0	2	2
2	AL/Lysine	80	16	2	2
3	AL/Lysine	40	8	2	2
4	AL/Lysine	20	4	2	2
5	AS Vehicle Control	0	0	2	2
6	Artesunate	400	80	2	2
7	Artesunate	200	40	2	2
8	Artesunate	100	20	2	2
9	Artesunate	50	10	2	2
10	AL/Lysine	320	64	2	2
11	AL/Lysine	160	32	2	2

Range-Finding (Phase 2)

Group ID	Formulation	Nominal Dose		Number of Rats	
		Level (mg/kg/day)	Concentration (mg/mL)	Males	Females
1	AL Vehicle Control	0	0	3	3
2	AL/Lysine	37.5	6	3	3
3	AL/Lysine	18.8	3	3	3
4	AL/Lysine	9.4	1.5	3	3
5	AL/Lysine	4.7	0.75	3	3
6	AS Vehicle Control	0	0	3	3
7	Artesunate	75	15	3	3
8	Artesunate	37.5	7.5	3	3
9	Artesunate	18.8	3.75	3	3
10	Artesunate	9.4	1.9	3	3
11	Arteether	25	50	3	3

Range-Finding (Phase 3)

Group ID	Formulation	Nominal Dose		Number of Rats	
		Level (mg/kg/day)	Concentration (mg/mL)	Males	Females
12	AL Vehicle Control	0	0	3	3
13	AL/Lysine	80	16	3	3
14	AS Vehicle Control	0	0	3	3
15	Artesunate	150	30	3	3

2.5.2.7 Dose Procedure: For the LD_{50} phase of the study, rats in Groups 1-9 were dosed on their respective Day 1 (September 26, 2001). As no mortality occurred for rats in dose Group 2 (80 mg/kg AL/lysine) on or before Day 5 of the study, rats in Groups 10 (160 mg/kg AL/lysine) and 11 (320 mg/kg AL/lysine) were subsequently dosed on their respective Day 1 (October 1, 2001). Each rat received a single iv injection of the designated dose of AL or AS (or the respective vehicle control), as indicated in the preceding table, via a tail vein; the dose volume administered was 5 mL/kg.

For the dose range-finding phases (Phases 2 and 3), each rat in Groups 1-10 and 14-15 received single daily iv doses, administered via a tail vein, of AL/lysine, AS, or the appropriate vehicle formulation, as indicated in the preceding tables, once daily for 7 consecutive days; rats in Group 11 received single daily im doses of AE, administered into the left or right hind leg, once daily for 7 consecutive days. Rats in Groups 12 and 13 were scheduled to receive single daily iv doses, administered via a tail vein, of AL/lysine or the appropriate vehicle formulation once daily for 7 consecutive days; however, due to necrotic tails, dosing of the rats in Groups 12 and 13 was discontinued after Day 5.

Note: After the histopathological evaluations of the Phase 3 animals had been completed, it was discovered that, during Phase 3, not all rats in the 80 mg/kg/day AL dose group (Group 13) had received an iv dose for 4 days, as previously reported. It was found that all 3 male and all 3 female rats in the 80 mg/kg/day AL dose group were successfully dosed iv on Days 1 and 2. The raw data indicated that, on Days 3, 4, and 5, the three male and two of the three female rats were dosed into the tail but not the tail vein; this mishap had occurred because after the first day of dosing, the tails of the rats given AL became swollen and subsequently turned black making it difficult to see the tail veins. One of the three female rats was successfully dosed iv on Days 1, 2, 4, and 5 but not on Day 3. The COR was contacted as soon as this error in reporting was discovered.

For Phase 2, the dose volume of AL/lysine or the appropriate vehicle formulation administered to rats in Groups 1-5 was 6.25 mL/kg, except on the first day of dosing for the males (October 24, 2001), when the dose volumes were calculated using a base volume of 5 mL/kg; the increase in the base dose volume from 5 mL/kg to 6.25 mL/kg occurred at the request of the Sponsor after dosing had been initiated on Day 1 for the male rats. The dose volume of AS or the appropriate vehicle formulation administered to rats in Groups 6-10 was 5 mL/kg, and

the dose volume of AE administered to rats in Group 11 was 0.5 mL/kg. For Phase 3, the dose volume administered to rats in Groups 12-15 was 5 mL/kg.

All doses in all three phases were administered using 3-cc plastic syringes (Becton-Dickinson and Co.; Franklin Lakes, NJ) fitted with 23.5-gauge needles (Sherwood Medical; St. Louis, MO).

2.5.2.8 Body Weights: For the LD_{50} phase, individual body weights for each rat were obtained during Week –1 (for randomization), on Day 1 (prior to dosing), and on Day 8. For the dose range-finding phase (Phases 2 and 3), individual body weights for each surviving rat were obtained during Week –1 (for randomization), on Days 1-7 prior to dosing, on Day 15, and prior to sacrifice/necropsy on Day 8 (Phase 3, Groups 12 and 13 only) or Day 23.

2.5.2.9 Clinical Observations: For all phases of the study, the rats were observed once daily during quarantine and twice daily during the study for signs of mortality or moribundity. During the LD_{50} phase, the rats were observed twice daily, morning and afternoon, at least 4 hours apart on Days 1-7, and once on Day 8 prior to sacrifice. These evaluations included an assessment for abnormal posture, activity, level of arousal, and breathing; the site of injection was monitored closely for irritation, discoloration, and/or swelling.

During the dose range-finding phase (Phases 2 and 3), each surviving rat was observed for detailed signs of toxicity twice daily on Days 1-7, at approximately 1 and 5 hours postdose; twice daily on Days 8-14, morning and afternoon, approximately 4 hours apart; once daily on Days 15-22; and prior to necropsy on Day 23; the site of injection was monitored closely for irritation and/or swelling or other adverse reactions. The following exceptions to the observation schedule occurred during Phase 3: On Day 2, the 5-hour postdose observation was not performed/recorded for rats in Group 13, with the Study Director's permission, due to the late completion of dosing for that group on that day; and, because dosing was discontinued after Day 5 for rats in Groups 12 and 13 due to the necrotic tails of the animals in Group 13, no observations were performed/recorded for rats in these dose groups on Days 6 and 7.

2.5.2.10 Clinical Pathology: Clinical pathology evaluations were not conducted during the LD_{50} phase. On Day 8 (Phase 3, Groups 12 and 13 only) or Day 15 of the dose range-finding phase (Phase 2 and 3), prior to scheduled necropsy, each rat was anesthetized with CO_2/O_2, and blood samples were obtained from the retro-orbital sinus of each rat into tubes containing EDTA (hematology samples) or no anticoagulant (clinical chemistry samples). Hematology and clinical chemistry determinations were performed on each blood sample, as described in Section 2.2.2.9.

2.5.2.11 Animal Disposition/Necropsy: For the LD_{50} phase, surviving rats were sacrificed on Day 8 by CO_2/O_2 asphyxiation. Carcasses were discarded without evaluation.

For the dose range-finding phase (Phases 2 and 3), with the exception of rats in Groups 12 and 13, all rats in all dose groups were anesthetized on Day 23 with ketamine/xylazine (75 mg/kg/10 mg/kg, administered ip), and sacrificed by exsanguination/perfusion. Because dosing was discontinued early due to necrotic tails, rats in Groups 12 and 13 were anesthetized and sacrificed on Day 8, following the same procedures. To achieve the perfusion, once each rat was

anesthetized, the thorax and pericardium were opened to expose the heart, a needle connected to perfusate was placed in the left ventricle, and the right atrium was opened to effect exsanguination euthanasia. Each rat was perfused with a chilled 0.9% sodium chloride solution, containing 1 unit of heparin per mL, for approximately 2 minutes; the heparinized saline perfusion was followed by perfusion with 500-700 mL of Bouin's solution. After perfusion, the carcass of each rat was maintained *in situ* for 30-120 minutes prior to dissection. The following tissues were examined at necropsy:

Adrenals (2)
Bone
Cecum
Colon
Duodenum
Epididymis
Esophagus
Eyes (2)
Fallopian tube
Gross lesions
Injection site (tail)
Heart
Ileum
Jejunum
Kidneys (2)
Liver
Lungs and bronchi
Lymph nodes (mesenteric)
Mammary gland (females only)
Marrow (femur)
Ovaries (2)
Pancreas
Pituitary
Prostate
Salivary gland
Sciatic nerve
Seminal vesicle
Skin
Skeletal muscle
Spleen
Stomach
Testes (2)
Thyroid/parathyroid (2)
Thymus
Tongue
Urinary bladder
Uterus (corpus and cervix)
Vagina

In that no gross lesions were observed (except for the tails of the Group 13 animals), no nonbrain tissues were saved and fixed. The brain was removed from each rat and immersed in fresh Bouin's fixative for at least 24 hours.

2.5.2.12 Histopathology. Each brain was processed as follows:

Clearing of picric acid (from Bouin's solution): Brains were immersed in 70% ethanol, and the ethanol was changed every 24 hours, until the brains were no longer colored with picric acid.

Dehydration, clearing, and embedding: Each brain was dehydrated in ascending grades of ethyl alcohol and then cleared with xylene. Individual brains were dissected as follows:

- A transverse cut was made at the spino-medullary junction, caudal to the dorsal column nuclei.
- A second transverse cut was made immediately caudal to the inferior colliculi.
- The cerebellum remained attached to this hindbrain block.

Subsequently, the tissues were embedded in paraffin. Only one brain section was embedded per paraffin block.

The midbrain and forebrain blocks were stored in 70% ethanol and then forwarded to the Sponsor.

Microtomy. Serial transverse sections were cut through the hindbrain at 10-µm increments. The sections were mounted on slides according to the following scheme: Sections #1 and 2 were mounted on the first glass slide for hematoxylin and eosin (H&E) staining. Sections #3 and 4 were mounted on another glass slide for Klüver-Barrera (K-B; luxol fast blue counterstained with cresyl violet) staining. Sections #5 and 6 were mounted on another glass slide for staining with the Nissl method (cresyl violet). Subsequently, sections #20 and 21 were mounted for H&E staining. Sections #22 and 23 were mounted on another slide for K-B staining. Sections #24 and 25 were mounted for Nissl (cresyl violet) staining. Thereafter, every 20^{th}-25^{th} section was mounted in this same sequence until the entire hindbrain/cerebellum block had been processed. The remaining serial sections that were cut were retained.

Sections were examined microscopically by a pathologist. For the brain tissues, lesions were graded for severity on a five-point scale with 0 as normal and 4 as the most severe. The study pathologist established criteria for each of these grades, and the severity criteria related to the number or percentage of neurons involved. The nuclear groups studied included the vestibular system [n. vestibularis medialis; n. vestibularis descendens (spinal)], the reticular formation (n. gigantocellularis; n. pontis centralis caudalis; n. pontis centralis oralis), and the auditory system (n. trapezoidalis; n. olivaris superior). Sections were subsequently forwarded to Dr. J.M. Petras at WRAIR for peer review.

2.5.2.13 Statistical Analyses. No statistical analyses were performed on data generated during the LD_{50} phase. For the dose range-finding phase (Phases 2 and 3), the group mean and standard deviation was calculated for each dose-sex group for body weights and the hematology and clinical chemistry parameters; these data were also subjected to statistical analysis by ANOVA, followed by Dunnett's test. In all cases, the lower limit for statistical significance was defined as $p<0.05$.

2.5.3 Results

2.5.3.1 LD_{50} Phase Results

Dose Formulation Analyses. The measured concentration of AL or AS in the individual dose formulations was within 10% of the theoretical concentration in all formulations, with one exception. For the 64 mg/mL AL formulation, the mean concentration of AL was 89.2% of theory. There was considerable variation in the measured concentration of AL in the three samples removed from the 64 mg/mL formulation, suggesting that error may have occurred during the sampling and dilution of the formulation for analysis. Due to technical oversight, the formulation was not re-analyzed at this point. The possibility of a sampling or technical error was supported by the observation that the concentration of AL in the 32 mg/mL formulation, which was prepared by dilution of the 64 mg/mL formulation, was determined to be 98% of theory.

Mortality. A summary of mortality for the LD_{50} phase of the study is presented in Table 33. Two of two female rats in the 160 mg/kg AL/lysine dose group and 2/2 male and 2/2 female rats in the 320 mg/kg AL/lysine dose group died on Day 1 within 30 minutes after dosing.

Two of two male and 2/2 female rats in the 400 mg/kg AS dose group were found dead on Day 2.

Clinical Observations. Prostration and/or hypoactivity were observed after dosing on Day 1 for 2/2 males in the 160 mg/kg AL dose group; these clinical signs were transient and were not observed at the second scheduled daily observation period which occurred approximately 4 hours after dosing. Other adverse clinical signs of toxicity noted for rats given AL were related to abnormalities in the tail of the animals. Black or dark discoloration of the tail was observed on Days 2-8 for 1/2 males and 2/2 females in the 80 mg/kg AL dose group and for 2/2 males in the 160 mg/kg AL dose group; in addition, partial loss of the tail occurred on Day 5 for one of the female rats in the 80 mg/kg AL dose group. Necrosis of the tail was also noted on Days 5-8 for 2/2 male rats in the 160 mg/kg AL dose group.

For rats given AS, the only adverse clinical sign noted was ataxia within the first 30 minutes after dosing on Day 1 for 1/2 males and 2/2 females in the 400 mg/kg AS dose group; the ataxia was transient and was not observed at the second scheduled daily clinical observation period which was conducted approximately 4 hours later.

Body Weights. A reduction in mean body weight gain, compared to the corresponding vehicle control rats, was observed for male and female rats in the 80 mg/kg AL dose group

between Days 1 and 8. Body weight loss (5% of mean value) was observed between Days 1 and 8 for male rats in the 160 mg/kg AL dose group; female rats in the 160 mg/kg AL dose group and male and female rats in the 320 mg/kg AL dose group died prior to Day 8.

The mean body weight gain observed between Days 1 and 8 for male and female rats given 50, 100, or 200 mg/kg of AS was similar to that observed for animals in the corresponding vehicle control group; the male and female rats given 400 mg/kg of AS died prior to the scheduled body weight measurements on Day 8.

2.5.3.2 Dose-Range Finding Phase (Phases 2 and 3) Results

Dose Analysis. Dose formulations containing AL at a nominal concentration of 0.75, 1.5, 3, 6, or 16 mg/mL were prepared for use during the two phases (Phase 2 and Phase 3) of the range-finding study. HPLC analyses conducted on the formulations prior to dosing indicated the concentration of AL in each formulation was 98.3 to 102% of the nominal (theoretical) concentration. The percent relative standard deviation (% RSD) for analyses of samples removed from the top, middle, or bottom of each formulation ranged from 0.16 to 1.2%, indicating that each formulation was homogenous. The postdose analyses conducted on the AL formulations prepared for use during Phase 2 established that the concentration of AL in each formulation was the same as at the time of preparation, indicating that the formulations had remained stable during the period of use.

Dose formulations containing AS at a nominal concentration of 1.9, 3.75, 7.5, 15, or 30 mg/mL were prepared for use during Phase 2 and Phase 3 of the range-finding study. Formulations were prepared daily on each day of dosing; HPLC analyses of individual formulations were performed on the formulations prepared for use on Days 1, 3, and 7 of Phase 2 and on Days 1 and 7 of Phase 3. The results of the HPLC analyses of these formulations indicated that the concentration of AS in each formulation was between 97.3 and 110% of the nominal (theoretical) concentration. The %RSD for analyses of samples removed from the top, middle, or bottom of each formulation was between 0.32 and 4.8, indicating that each formulation was homogenous.

A single dose formulation of AE, containing a nominal concentration of 50 mg/mL of AE, was prepared for use during Phase 2. The measured concentration of AE in the formulation was 103% of the nominal (theoretical) concentration. The %RSD for analyses of samples removed from the top, middle, or bottom of the formulation was 0.53, indicating the formulation was homogenous.

Mortality. No mortality was observed for male and female rats given iv doses of AL of 4.7, 9.4, 18.8, or 37.5 mg/kg/day for 7 consecutive days or 80 mg/kg/day for ≤4 days.

No mortality was observed for male and female rats given iv doses of AS of 9.4, 18.8, 37.5, or 75 mg/kg/day for 7 consecutive days or for male rats given 150 mg/kg/day for 7 consecutive days; 1/3 female rats given 150 mg/kg/day of AS was found dead on Day 6.

No mortality was observed for male or female rats given an im dose of AE of 25 mg/kg/day for 7 consecutive days.

Clinical Observations. No adverse clinical signs were observed for male or female rats given iv doses of 0, 4.7, 9.4, or 18.8 mg/kg/day of AL for 7 consecutive days. Adverse clinical signs observed for rats given 37.5 mg/kg/day of AL were limited to abnormalities of the tail and included swelling of the tail in one or more male or female rats on multiple days between Days 2 and 13; in addition, a sore or ulcer was observed on the tail of 1/3 male and 1/3 female rats between Days 8-12 or Days 5-16, respectively. For rats given 80 mg/kg/day of AL for ≤4 days, hunched posture was noted for 3/3 male and 3/3 female rats on one or more days between Days 4 and 8; one of three female rats in this dose group also appeared emaciated on Day 8. Other adverse clinical signs observed for rats given 80 mg/kg/day of AL were restricted to the tail and included: black or dark tail in 3/3 male (Days 3-4) and 1/3 (Day 1) or 2/3 female (Days 3-4) rats, and tail swelling and/or necrosis in 3/3 males (Day 5) and 2/3 females (Days 4, 5, and 8). Due to the development of a dark colored or swollen tail, by Day 5 it had become virtually impossible to see a vein in the tail of all animals in the 80 mg/kg/day AL dose group and dosing was discontinued after this day for animals in this dose group and the corresponding vehicle control group.

No adverse clinical signs were observed for male and female rats given iv doses of 0, 9.4, 18.8, or 37.5 mg/kg/day of AS for 7 consecutive days. One of three male rats in the 75 mg/kg/day AS dose group displayed a swollen tail on Days 9-12. No adverse clinical signs were observed for male rats given 150 mg/kg/day of AS for 7 consecutive days. The female rat in the 150 mg/kg/day AS dose group that was found dead on Day 6 displayed diarrhea, a nasal discharge, and hunched posture for 1 or 2 days preceding death. The other two female rats in the 150 mg/kg/day AS dose group displayed diarrhea, emaciation, a nasal discharge, hunched posture, and wet/skin fur in the abdominal/vulvular region on 2 or more days between Days 3 and 8; in addition, a sore/ulcer was noted on the tail of each of these two rats on Days 17 and 18.

No adverse clinical signs were observed on any day during the study for male or female rats given an im dose of 25 mg/kg/day of AE for 7 consecutive days.

Body Weights. Group mean body weights for animals dosed during Phases 2 and 3 are summarized in Table 34. Male and female rats given 4.7, 9.4, 18.8, or 37.5 mg/kg/day of AL daily for 7 consecutive days gained weight throughout the study and their rate of weight gain was similar to that observed for rats in the corresponding vehicle control group. Body weight loss was observed on multiple days between Day 1 and Day 8 for male and female rats given 80 mg/kg/day of AL for ≤4 days. The greatest decrease in body weight was observed on Day 6 for male rats and on Day 5 for female rats where the mean body weight of the animals was 82.8% and 87.9%, respectively, of the corresponding Day 1 mean body weight.

Male rats given 9.4, 18.8, 37.5, or 75 mg/kg/day of AS and female rats given 9.4, 18.8, or 37.5 mg/kg/day of AS, daily for 7 consecutive days, gained comparable amounts of body weight during the study to the male and female rats in the corresponding vehicle control group. Decreased body weight gain, compared to the corresponding vehicle control group rats, and/or body weight loss was observed during the dosing period for male rats in the 150 mg/kg/day AS

dose group and for female rats in the 75 and 150 mg/kg/day AS dose groups. The greatest body weight loss was observed on Day 5 for female rats in the 150 mg/kg/day AS dose group, where the mean body weight of the animals was 89.4% of Day 1.

Male and female rats given 25 mg/kg/day of AE, daily for 7 consecutive days, gained weight throughout the study and their rate of body weight gain was comparable to that of the animals in the AL and AS vehicle control groups that were dosed during Phase 2.

Hematology. Group mean hematology values are summarized in Table 35. Group mean values were compared to the corresponding group mean vehicle control values; group mean values for rats in the AE dose group were compared to the AS vehicle control group as there was no comparable vehicle control group. Individual values from rats in all AL, AS, and AE dose groups were also compared to the range of values that was observed for rats in all the vehicle control groups as no appreciable differences in clinical pathology parameters were observed between the different vehicle control groups. Statistical significance was considered; however, the small group size limited the value of these findings. Differences in group means were considered biologically relevant if they were greater than those differences expected to be due to analytical or interindividual variability.

A summary of the results is as follows: Hematology changes observed on Day 8 that were associated with administration of 80 mg/kg/day of AL included increases in group mean WBC values, reticulocyte percents or counts (male only), neutrophil counts, lymphocyte counts, monocyte counts, and platelet counts (female only), and mild decreases in RBC, HGB, and HCT values. Minimal increases in group mean or individual reticulocyte counts or percents that were observed on Day 15 for male and female rats in the 9.7, 18.8, and 37.5 mg/kg/day AL dose groups were also considered potentially drug related but of limited toxicological relevance due to the lack of appreciable changes in erythrocyte parameters. Hematology changes observed on Day 15 that were associated with administration of 150 mg/kg/day of AS were only observed in female rats and included increases in group mean MCV, reticulocyte percent, reticulocyte count, neutrophil and monocyte values, and decreases in group mean RBC, HGB, and HCT values. A mild increase in the group mean neutrophil count that was observed on Day 15 for female rats in the 75 mg/kg/day AS dose group was also considered to be potentially drug related. Hematology changes observed on Day 15 that were associated with administration of 25 mg/kg/day of AE were limited to minimal increases in the group mean reticulocyte count and percent which were observed in male but not female rats.

Artelinate. Group mean WBC counts that were observed for male and female rats in the 80 mg/kg/day AL dose group on Day 8 were mildly increased with values that were 2.0- and 2.1-fold greater than the values observed for rats in the vehicle control group. Mild (2.0- to 3.9- fold) to moderate (4.0- to 5.9-fold) increases in the group mean neutrophil counts were observed for male and female rats in the 80 mg/kg/day AL dose group on Day 15 with values that were 3.8- and 5.1-fold, respectively, greater than the values observed for rats in the respective vehicle control group. Increased group mean monocyte counts were also observed for male and female rats in the 80 mg/kg/day AL dose group with values that were 2.3- and 4.7-fold greater than the values observed for rats in the respective vehicle control group. Minimal increases in lymphocyte counts

were also observed for male and female rats in the 80 mg/kg/day AL dose group with values that were 1.6- and 1.7-fold greater than the values observed for rats in the vehicle control dose group. An increase in the group mean platelet count (1.9-fold greater than vehicle control value) was also observed on Day 8 for female rats in the 80 mg/kg/day AL dose group.

Decreased group mean RBC, HGB, and HCT values were observed for male and female rats in the 80 mg/kg/day AL dose group on Day 8; group mean RBC values were 84% and 79%, respectively, of the values observed for rats in the respective vehicle control group.

A trend for minimal increases (1.5- to 1.9- fold greater than control values) in the group mean reticulocyte percent or counts was observed for male and female rats in the 9.7,18.8, and 37.5 mg/kg/day AL dose groups on Day 15 and for male rats in the 80 mg/kg/day AL dose groups on Day 8. In both male and female rats, at least 2/3 rats in each of these dose groups were observed to have reticulocyte count or percent values that were greater than the maximal values observed for rats in all vehicle control groups. While the predominance of individual rats with reticulocyte values outside the range of values observed for vehicle control rats was consistent with a drug-related response, the lack of appreciable changes in erythrocyte parameters suggested that the change was of limited toxicological relevance except for male rats in the 80 mg/kg/day AL dose group.

Clinical observations of tail swelling and tail necrosis that were noted for rats in the 80 mg/kg/day AL dose groups were consistent with findings of leukocytosis, neutrophilia, and monocytosis. While decreases in RBC, HGB, and HCT values may occur secondarily to body weight loss or decreased body weight gain in rats, the combination of decreased erythrocyte values observed in rats in the 80 mg/kg/day AL dose group and the presence of mild to moderate increases in group mean reticulocyte values in rats in the 9.7 to 37.5 mg/kg/day AL dose groups and in male rats in the 80 mg/kg/day AL dose group were consistent with a drug-related effect on hematopoiesis.

Artesunate. No biologically relevant differences in the group mean WBC counts were observed for rats in the AS dose groups; however, an increased individual WBC count (greater than the maximum value observed for rats in all vehicle control groups) was observed in 1/2 female rats in the 150 mg/kg/day AS dose group on Day 15. Increased group mean neutrophil counts were observed for female rats in the 75 and 150 mg/kg/day AS dose groups on Day 15, with values that were 2.1- and 3.8-fold greater than the values observed in rats in the vehicle control group. Monocyte counts were increased in 1/2 male rats in the 150 mg/kg/day AS dose group.

Decreased group mean RBC, HGB, and HCT values were also observed for female rats in the 150 mg/kg/day AS dose group with a RBC value that was 79% of the value observed for rats in the vehicle control group. The group mean MCV value that was observed for female rats in the 150 mg/kg/day AS dose group was increased, with a value that was 108% of the value observed for rats in the respective vehicle control group.

Administration of 150 mg/kg/day of AS was associated with mild (2.0- to 3.9-fold) to moderate (4.0- to 5.9-fold) increases in group mean reticulocyte percent and counts on Day 15 in female rats, with values that were 4.2- and 3.4-fold greater than the values observed for rats in the vehicle control group. Minimal increases (1.8- and 1.6-fold greater than control values) in group mean reticulocyte percent or counts that were observed for male rats in the 18.8 mg/kg/day AS dose group were considered to be not clearly compound related or not of toxicological relevance due to the lack of a dose relationship, the minimal magnitude of the change, and the lack of changes in RBC, HGB, and HCT.

Clinical observations of tail swelling and tail necrosis that were noted for rats in the 150 mg/kg/day AS dose group were consistent with findings of neutrophilia and monocytosis. While decreases in RBC, HGB, and HCT values may occur secondarily to body weight loss or decreased body weight gain in rats, the combination of decreased erythrocyte values, increased MCV, and the presence of mild to moderate increases in group mean reticulocyte values for female rats in the 150 mg/kg/day AS dose group were consistent with a drug-related effect on hematopoiesis.

Arteether. Hematology changes associated with administration of 25 mg/kg/day of AE were limited to minimal increases in group mean reticulocyte percents and counts in male rats on Day 15, with values that were 1.9- and 1.8-fold greater than the values observed for rats in the AS vehicle control group. All male rats in the AE dose group were observed to have individual reticulocyte percents and counts that were greater than the maximum value that was observed for rats in the vehicle control group. Female rats in the 25 mg/kg/day AE dose group were also observed to have slight increases in group mean reticulocyte percent and count; however, the differences were not considered biologically relevant due to the minimal magnitude of the increases.

Clinical Chemistry. Group mean clinical chemistry values are summarized in Table 36. Group mean values were compared to the corresponding group mean vehicle control values; group mean values for rats in the AE dose group were compared to the AS vehicle control group as there was no comparable vehicle control group. Individual values from rats in all AL, AS, and AE dose groups were also compared to the range of values that was observed for rats in all the vehicle control groups as no appreciable differences in clinical pathology parameters were observed between the different vehicle control groups. Statistical significance was considered; however, the small group size limited the value of these findings. Differences in group means were considered biologically relevant if they were greater than those differences expected to be due to analytical or interindividual variability.

A summary of the results is as follows: Decreases in group mean total protein (female), albumin, and A/G ratio values, and increases in group mean globulin values were associated with administration of 80 mg/kg/day of AL. Decreases in the group mean albumin value and A/G ratio and increases in the group mean globulin value were also associated with administration of 150 mg/kg/day of AS in female rats. No drug-related differences in clinical chemistry parameters were associated with administration of 4.7, 9.7, 18.8, or 37.5 mg/kg/day of AL, 9.4, 18.8, 37.5, or 75 mg/kg/day of AS, or 25 mg/kg/day of AE.

Artelinate. The group mean total protein value that was observed on Day 8 for female rats in the 80 mg/kg/day AL dose group was decreased with a value that was 93% of the value observed for rats in the vehicle control group. Three of three rats in the AL dose group were observed to have total protein values that were less than the range of values observed for rats in all of the vehicle control groups. The group mean albumin values that were observed for male and female rats in the 80 mg/kg/day AL dose group on Day 8 were decreased with values that were 77% and 74%, respectively, of the values observed for rats in the corresponding vehicle control group. All rats in this dose group were observed to have albumin values that were less than the minimum value observed for rats from all of the vehicle control groups. Increases in the group mean globulin value were observed for male and female rats in the 80 mg/kg/day AL dose group on Day 8 with values that were 1.4- and 1.3-fold, respectively, greater than the values observed for rats in the vehicle control group. Decreases in the group mean A/G ratios were also observed for male and female rats in the 80 mg/kg/day AL dose group on Day 8 with values that were 58% and 57%, respectively, of the value observed for rats in the vehicle control group.

The pattern of decreases in albumin and A/G ratios and increases in globulin values was consistent with changes associated with inflammation and was consistent with clinical observations of tail swelling and necrosis that were noted for rats in the 80 mg/kg/day AL dose group. The decreases in group mean total protein, albumin, and A/G ratios and increases in group mean globulin values were considered to be biologically relevant and drug related as the pattern was consistently observed for rats in the high-dose group.

The group mean BUN and creatinine values that were observed for female rats in the 80 mg/kg/day AL dose group on Day 8 were increased, with values that were 140% and 138%, respectively, of the values observed for rats in the vehicle control group. On review of individual data, 1/3 and 2/3 of the rats in this dose group were observed to have BUN or creatinine values, respectively, that were greater than the maximum values observed for rats in all control groups. The increases in BUN and creatinine were not clearly drug related; these changes were probably secondary to the condition of the rats at the time of sacrifice on Day 8 and the concurrent tail vein necrosis, tail swelling, and hunched posture noted on this day.

Other statistically significant findings, including increases in group mean glucose (female, 4.7 mg/kg/day of AL, Day 15) and chloride (female, 4.7 and 18.8 mg/kg/day of AL, Day 15) and decreases in ALT (9.7 mg/kg/day of AL, Day 15) were not considered to be biologically relevant or drug related due to the minimal magnitude of the responses, the lack of a dose relationship, and the presence of data for individual animals that were generally comparable to control data.

Artesunate. The group mean albumin and A/G values that were observed for female rats in the 150 mg/kg/day AS dose group on Day 15 were decreased with values that were 76% and 49%, respectively, of the values observed for rats in the corresponding

vehicle control group. All female rats in this dose group were observed to have albumin values that were less than the minimum value observed for rats from all of the vehicle control groups. Increases in the group mean globulin values were observed for female rats in the same dose group, with a group mean globulin value that was 1.6-fold greater than the value observed for rats in the vehicle control group. The pattern of decreases in albumin and A/G ratios and increases in globulin values that were observed for female rats in the 150 mg/kg/day AS dose group was consistent with changes associated with inflammation. The decreases in group mean total protein, albumin, and A/G ratios and increases in group mean globulin values were considered to be drug related and consistent with clinical observations of tail sores/ulcers and diarrhea.

Arteether. No chemistry changes were associated with administration of 25 mg/kg/day of AE to rats for 7 consecutive days. The group mean albumin value that was observed on Day 15 for female rats in the 25 mg/kg/day AE dose group was 91% of the value observed for rats in the AS vehicle control group; however, this change was probably not drug related as only 1/3 of the rats in this dose group had an albumin value that was less than the range of values observed for rats in the control group.

Macroscopic and Microscopic Pathology. During Phase 2 and Phase 3, no gross lesions were observed for male or female rats in any AL, AS, or AE dose groups, with the exception of the discoloration and/or necrosis of the tail observed for rats in the 80 mg/kg/day AL dose group.

Microscopic pathology was conducted only on the hindbrain of rats dosed during Phases 2 and 3. There were no microscopic lesions in the hindbrain of rats that were related to treatment with AL at doses up to 80 mg/kg/day for ≤4 days or to treatment with AS at doses up to 150 mg/kg/day for 7 consecutive days. The hindbrain of all animals in the AL or AS dose groups that were examined was within normal limits.

For animals in the 25 mg/kg/day AE dose group, no lesions were noted in the hindbrains of male rats but lesions were observed in 1/3 female rats. The degenerative lesions that were noted in the female rats consisted of neuronal degeneration of 2 to 4 cells per section in the trapezoid nucleus.

3.0 KEY RESEARCH ACCOMPLISHMENTS

- Characterized the pharmacokinetics of artelinic acid and radioactivity in dogs given iv or po doses of [^{14}C]artelinic acid.
- Determined the oral bioavailability of artelinic acid and radioactivity derived from [^{14}C]artelinic acid in dogs.
- Determined the rate and the routes of excretion of radioactivity following iv or po administration of [^{14}C]artelinic acid to dogs.
- Characterized urinary metabolites of artelinic acid.
- Determined the toxicological effects of 14-day administration of oral suspensions of artelinic acid to rats.
- Characterized the toxicological effects of 14-day intramuscular administration of arteether to rats.
- Determined a "no effect" dose for the production of the neurotoxicity by artelinic acid in rats given daily po doses for 14 days.
- Characterized the toxicological effects of 14-day administration of oral suspensions of artelinic acid to dogs.
- Characterized the toxicological effects of 14-day intramuscular administration of arteether to dogs.
- Determined a minimum "no effect" dose for the production of neurotoxicity by artelinic acid in dogs.
- Characterized the pharmacodynamic effects of an injectable formulation of artelinic acid/lysine in dogs given a single iv dose, equal to or greater than the anticipated clinical dose, daily for 7 consecutive days.
- Determined the toxicological effects and maximum tolerated dose of artelinic acid in rats given daily iv doses of an artelinic acid/lysine formulation for up to 7 days.
- Determined the toxicological effects and maximum tolerated dose of artesunic acid in rats given daily iv doses for 7 consecutive days.
- Characterized the toxicological effects of arteether in rats given daily intramuscular doses for 7 consecutive days.
- Characterized the histopathological lesions produced in the brainstem of rats given a daily im dose of arteether for 7 consecutive days.
- Determined the "no effect" dose for the production of histopathological lesions in the brainstem of rats given a daily iv doses of an artelinic acid/lysine salt formulation for 7 consecutive days.
- Determined the "no effect" dose for the production of histopathological lesions in the brainstem of rats given a daily iv doses of artesunic acid for 7 consecutive days.

4.0 REPORTABLE OUTCOMES

Bossone, C.A., Q. Li, S. Mog, P. Lee, C. Ohrt, H. Chung, P.E. Noker, J.G. Page, and R. Brueckner. Toxicity of 14-day oral administration of artelinic acid. Abstract #311. 48th Annual Meeting of the American Society of Tropical Medicine and Hygiene, 1999.

5.0 CONCLUSIONS

During the initial study conducted under the contract, information was obtained on the pharmacokinetics, bioavailability, excretion, and metabolism of artelinic acid in dogs given single iv or po doses of [^{14}C]artelinic acid. Following iv administration of approximately 10 mg/kg of [^{14}C]artelinic acid to two male and two female dogs, the concentration of radioactivity in plasma during the first 30 minutes after dosing was higher than the corresponding concentration in whole blood. With time after dosing, however, the plasma concentration of radioactivity in individual dogs approximated the concentration in whole blood, indicating that equal distribution of radioactivity between plasma and red blood cells occurred over time. Consistent with this, the terminal half-life of radioactivity in plasma, as determined from non-compartmental pharmacokinetic analysis of the plasma radioactivity data, was 125 hours, which was similar to the half-life of radioactivity in whole blood. The results of LC/MS analyses of plasma samples demonstrated that, even at the early time points after administration of [^{14}C]artelinic acid, not all of the radioactivity in plasma was present as unchanged artelinic acid, indicating that artelinic acid was rapidly metabolized following iv administration to dogs. The estimated half-lives for artelinic acid in plasma were 0.11 and 0.64 hours. Thus, the terminal half-life of artelinic acid in plasma was substantially shorter than that for total radioactivity indicating that the radiolabeled metabolites of [^{14}C]artelinic acid were eliminated from plasma at a much slower rate than was unchanged artelinic acid.

Similarly, for dogs administered a po dose of [^{14}C]artelinic acid, the concentration of radioactivity in plasma was higher than the corresponding concentration of radioactivity in whole blood through 8 hours after dosing; at later times after dosing, the levels of radioactivity in plasma approximated the corresponding level in whole blood, as observed in dogs given an iv dose. Unchanged artelinic acid was quantifiable in plasma collected through 4-8 hours after po administration of [^{14}C]artelinic acid; however, the concentration of artelinic acid in plasma at any given time was lower than the corresponding concentration of radioactivity. Thus, as observed after iv dosing, artelinic acid was metabolized after po administration. The estimated mean half-life of artelinic acid in dogs given a po dose was 0.73 hours.

Based on AUC values calculated from concentrations of radioactivity in whole blood, the oral bioavailability of radioactivity derived from [^{14}C]artelinic acid was estimated to be 85%; based on concentrations of radioactivity in plasma, the oral bioavailability was calculated to be 88%. Thus, radioactivity derived from [^{14}C]artelinic acid was well absorbed by dogs after oral administration of the compound. The oral bioavailability of unchanged artelinic acid was determined to be 67%. These data further confirmed that not all absorbed radioactivity was intact [^{14}C]artelinic acid, indicating that the compound had been metabolized prior to and/or after absorption.

Urinary and fecal elimination data obtained after iv administration of [^{14}C]artelinic acid to dogs demonstrated that a mean value of 37.2% of the dose was eliminated in urine and 47.3% in feces within 192 hours after dosing; radioactivity in the cage rinses, which may have been derived from either urine or feces, accounted for 8.8% of the dose. The appearance of radioactivity in feces after iv administration indicated that a portion of the dose of [^{14}C]artelinic

acid underwent biliary excretion. After po administration of [^{14}C]artelinic acid, a slightly lower percentage of the dose was eliminated in urine (mean = 25.5%) and a slightly higher percentage was excreted in feces (57.5%) than after iv administration; the amount recovered in the cage rinses was similar to what was observed with the iv dose. The lower urinary excretion of radioactivity by dogs given an oral dose was consistent with the whole blood radioactivity data which indicated that the oral bioavailability of radioactivity derived from [^{14}C]artelinic acid was less than 100%.

Chromatographic analyses of urine samples demonstrated that detectable levels of unchanged artelinic acid were not excreted in urine after either iv or po administration of [^{14}C]artelinic acid to dogs. These data were consistent with the short half-life of artelinic acid in plasma after either route of administration. Further, the radiolabeled metabolites excreted in urine were qualitatively and quantitatively similar after iv or po administration of [^{14}C]artelinic acid. These results demonstrated that [^{14}C]artelinic acid was extensively metabolized by dogs and that the degree of metabolism was independent of the route of administration. Data obtained during the metabolite analyses of urine also indicated that artelinic acid derivatives underwent conjugation reactions, consistent with what has been observed by other investigators (2,3). Thus, both Phase I and Phase II reactions appear to be involved in the metabolism of artelinic acid in dogs.

Chromatographic analyses also showed that the metabolite profiles of feces excreted after either iv or po administration of [^{14}C]artelinic acid to dogs were qualitatively and quantitatively similar. After either route of administration of [^{14}C]artelinic acid, no unchanged artelinic acid was detectable in feces.

In a companion study, we previously investigated the pharmacokinetics, bioavailability, distribution and metabolism of [^{14}C]artelinic acid in rats given an iv or po dose of 10 mg/kg.[13] In rats, the oral bioavailability of radioactivity derived from [^{14}C]artelinic acid was determined to be approximately 70%, a value which was slightly lower than that observed in the present study with dogs. In addition, in rats, the oral bioavailability of unchanged artelinic acid was lower than in dogs and was only approximately 28%. These apparent differences between rats and dogs in the oral bioavailability of radioactivity derived from [^{14}C]artelinic acid and of unchanged artelinic acid may have been related to a greater degree of gastrointestinal metabolism of artelinic acid in rats than in dogs or to a higher extent of first pass metabolism in the rat. Differences were also apparent between dogs and rats in the urinary and fecal excretion of radioactivity after iv or po administration of [^{14}C]artelinic acid. In rats, the percentages of the dose eliminated in urine and feces were the same after either route of administration of artelinic acid. In contrast, in dogs, a higher percentage of the dose was eliminated in urine after iv administration than after po administration of [^{14}C]artelinic acid. These data would suggest that a species difference may exist in the metabolism of artelinic acid.

In a subsequent study, the dose related pharmacodynamic effects of artelinic acid were determined in rats given daily po doses for 14 consecutive days. These investigations included an assessment of the clinical as well as histopathological toxicity of artelinic acid, with particular emphasis on the neurotoxicity of the compound. Arteether, which has been demonstrated to be

neurotoxic in several laboratory species,[14,15] served as the positive control agent for the assessment of drug-related neurohistopathological changes.

The results of this evaluation indicated that no mortality, no overt clinical signs of toxicity, and minimal effects on body weight gain were observed for rats given daily doses of artelinic acid of 80 mg/kg/day or lower for 14 consecutive days. At a higher dose level (320 mg/kg/day), however, clinical signs of toxicity, including emaciation, dehydration, hypoactivity, tics, body weight loss, and mortality were observed. Thus, the maximum tolerated dose of artelinic acid for rats given daily oral gavage doses for 14 consecutive days was determined to be 80 mg/kg/day.

Potential drug-related hematological changes were observed on Day 21 for rats given 14 consecutive daily oral gavage doses of artelinic acid of 20, 40, 80, or 320 mg/kg/day but not for rats given 14 consecutive daily im doses of arteether of 12.5 mg/kg/day. The hematological changes included increases in group mean reticulocyte counts for male and/or female rats given doses of artelinic acid of 20 to 320 mg/kg/day, mild but potentially biologically relevant increases in erythrocyte MCV values for rats given doses of artelinic acid of 20 or 80 mg/kg/day, and minimal decreases in mean RBC values for rats in the 80 mg/kg/day dose group. Thus, when given daily by gavage for 14 consecutive days, a dose of 10 mg/kg/day was a "no effect" dose of artelinic acid for the production of hematological changes. The dose of artelinic acid required to produce biologically relevant changes in the measured clinical chemistry parameters appeared to be higher than that which produced hematological changes. Only male rats in the 320 mg/kg/day artelinic acid dose group were observed to have a drug-related change in clinical chemistry parameters. This consisted of a decrease in ALP activity. The two female rats in the 320 mg/kg/day dose group died prior to the scheduled clinical pathology evaluations on Day 21.

Neurohistopathological lesions, which were predominantly characterized by neuron degeneration in the trapezoid nucleus and hind brain, were observed in rats given artelinic acid at dose levels of 40 mg/kg/day and higher. The neuron degeneration was characterized by loss of Nissl staining, swelling, and margination of the nucleus. Effected cells appeared to have red/brown cytoplasm on staining with H&E. The more severe lesions were observed for rats in the 320 mg/kg/day dose group. The severe lesions consisted of clumping of eosinophilic debris in some cells, with minimal evidence of satellitosis and minimal gliosis. Thus, when given daily by gavage for 14 consecutive days, the "no effect" dose of artelinic acid for the production of neurotoxicity in rats was 20 mg/kg/day. Neuron degeneration was also observed for male and female rats given 12.5 mg/kg/day of arteether. The histopathological lesions observed for rats given intramuscular arteether were consistent with the previously reported neurotoxicity of this compound. [14,15]

In a companion study, the dose related pharmacodynamic effects of artelinic acid were investigated in dogs given daily po doses for 14 consecutive days. During this investigation, an assessment of the clinical as well as histopathological toxicity of artelinic acid, with particular emphasis on the neurotoxicity of the compound, was made. Similar to the study conducted in rats, arteether served as the positive control agent for the assessment of drug-related neurohistopathological changes.

The results of this preliminary study indicated that no drug-related mortality was observed for dogs given artelinic acid as an oral suspension for 14 consecutive days at doses of 320 mg/kg/day and lower. No body weight losses were observed for male or female dogs given 20, 40, or 80 mg/kg/day of artelinic acid. In addition, no overt clinical signs of toxicity were observed, with the exception of sporadic diarrhea. Diarrhea was noted on various days between Day 2 and Day 21 of the study at all artelinic acid dose levels administered (20 to 320 mg/kg/day). The absence of a dose- or time-related incidence suggested the diarrhea was not directly drug related; in addition, no intestinal lesions were observed at necropsy in any dogs dosed with artelinic acid. Hypoactivity was observed beginning on Day 11 of the 14-day dosing period in one dog given 320 mg/kg/day of artelinic acid. Thus, only limited clinical signs of toxicity were observed for dogs given repeated daily doses of artelinic acid for 14 consecutive days. In contrast, male and female dogs given arteether displayed more overt clinical signs of toxicity. These signs included body weight loss and behavioral changes, such as ataxia, prostration, excessive salivation, tremors, and extreme hyperactivity, which were suggestive of neurological toxicity. The occurrence of generalized tremor and jerking limb movements have been reported previously in rats and monkeys given arteether; these expressions have been attributed to brainstem injury.[15,16]

An assessment of various clinical pathological parameters indicated that oral administration of artelinic acid to dogs at dose levels of 20 to 320 mg/kg/day or im administration of arteether (20 mg/kg/day) for 14 consecutive days resulted in potentially drug-related hematological changes. These changes included decreases in RBC, HGB, HCT, and reticulocyte counts on Day 7 or 15 and increased reticulocyte counts on Day 21. In that these changes were observed at all dose levels of artelinic acid administered, a "no effect" dose of artelinic acid for the production of hematological changes could not be established from the data. The dose of artelinic acid required to produce biologically significant changes in the measured clinical chemistry parameters appeared to be higher than that which produced hematological changes. Only female dogs given 80 or 320 mg/kg/day of artelinic acid were observed to have drug-related changes in clinical chemistry parameters. The predominant changes observed in these animals, and also in dogs given arteether, were decreased albumin and A/G ratios and/or increased globulin concentrations. In addition, dogs in the 20 mg/kg/day arteether dose group, but not dogs in any of the artelinic acid dose groups, displayed moderate increases in ALP, AST, LDH, and/or CK activity during the course of the 14-day dosing period.

An evaluation of plasma concentrations of artelinic acid in individual dogs indicated that plasma concentrations of artelinic acid increased in an apparent linear manner with increasing dose. No sex-related differences in plasma concentrations of artelinic acid were apparent. At all dose levels of artelinic acid given (20, 40, 80, and 320 mg/kg/day), the concentration of artelinic acid in plasma did not increase with repeated daily oral administration of the compound; plasma concentrations of artelinic acid in individual dogs were the same on Day 1 and Day 14 of dosing. This was consistent with the observation that the pharmacokinetics of artelinic acid in plasma were similar on the first and last day of the 14-day dose period. Thus, the pathways responsible for the metabolism/elimination of artelinic acid did not appear to become saturated with repeated administration of artelinic acid to dogs at oral dose levels up to 320 mg/kg/day.

In contrast, for male and female dogs given daily im doses of 20 mg/kg/day of arteether, plasma concentrations of arteether increased with successive days of arteether administration. On Day 14 of the dose period, peak plasma concentrations of arteether were approximately 3- to 4-fold higher than the concentration observed on Day 1. These data provided evidence for the accumulation of arteether in plasma with repeated daily administration.

For male and female dogs given from 20 to 320 mg/kg/day of artelinic acid, no histopathological lesions were observed in the brain or other tissues of any animal. Thus, a highest "no effect" dose of artelinic acid for the production of neurohistopathological and other histopathological toxicity in dogs could not be determined from the data. In contrast, neuron degeneration was observed in the nuclei of the hind brain of dogs given arteether. The neuropathological lesions observed in dogs given arteether were consistent with the previously reported neurotoxicity of this compound.[14-16]

Artelinic acid has been proposed as a likely drug for parental administration as an anti-malarial agent because of its chemical stability and aqueous solubility. The results of the study with iv artelinic acid in dogs indicated that an injectable formulation of artelinic acid/lysine was well tolerated by dogs given a single iv dose, equal to or greater than the anticipated clinical dose, for 7 consecutive days. This dosing schedule did not produce discernible venulitis in the peripheral leg vein of individual dogs into which it was injected or any other observable signs of clinical toxicity. These results provided support for further work related to the development of an iv formulation of artelinic acid for clinical administration.

In a subsequent investigation, the pharmacodynamics of artelinic acid was investigated in rats given daily iv doses of the artelinic acid/lysine formulation for up to 7 days. In this study, the pharmacodynamics of artelinic acid was assessed in rats through the monitoring of signs of clinical and pathological toxicity. The effects of artelinic acid were compared with those of two artemisinin derivatives, artesunic acid and arteether. Artesunic acid is currently marketed in China for the treatment of malaria. Arteether, which also has anti-malarial properties, served as the positive control in that it has demonstrated neurotoxicological activity.[14-16]

During this study, for rats given single daily iv doses, mortality was observed at AL doses of ≥160 mg/kg and at an AS dose of 400 mg/kg. Thus, it was determined that the maximum tolerated dose of AL for rats given a single iv dose was 80 mg/kg and the maximum tolerated dose of AS for rats given a single iv dose was 200 mg/kg.

Following 7 consecutive days of iv dose administration, no mortality was observed for male and female rats given ≤37.5 mg/kg/day of AL (total dose: ≤262.5 mg/kg) or ≤75 mg/kg/day of AS (total dose: ≤525 mg/kg). Due to the difficulty encountered during the iv administration of AL, no conclusions could be drawn relative to the lethality produced by higher multiple iv doses of AL.

The results of this study indicated that one of the significant clinical signs of toxicity produced by iv administered AL was a black and/or dark discoloration of the tail, which in some instances was accompanied by necrosis. In that the iv doses were administered into a tail vein, the occurrence of tail discoloration/necrosis indicated that AL had a direct adverse effect on the

vein and/or the surrounding tissue into which the dose was administered. Tail discoloration/necrosis was observed for rats given either single or multiple doses of AL. The occurrence of tail vein necrosis appeared to be dose dependent, suggesting that the venotoxicity produced by AL may have been a function of the concentration of AL in the individual dose formulations that were administered. Adverse effects on the tail vein/tail tissue were not observed for rats given single or multiple iv doses of AS.

Additional clinical signs of toxicity observed for rats given multiple doses of AL, and also for rats given multiple doses of AS or AE, included clinical pathological changes. Although rats in the 80 mg/kg/day AL dose group received only ≤4 iv doses of AL, clinical pathological changes were noted animals in this dose group. The changes that were observed on Day 8 for rats in this dose group and which were considered to be of possible toxicological significance included increases in group mean WBC values, reticulocyte percents or counts, neutrophil counts, lymphocyte counts, monocyte counts, platelet counts, and globulin values, and decreases in RBC, HGB, and HCT, total protein, albumin, and A/G ratio values. The increases in RBC, HGB, and HCT values, in combination with the presence of mild to moderate increases in reticulocyte values, suggested that AL may have effected hematopoiesis. The decreases in albumin and A/G ratios and increases in globulin values, as well the observed leukocytosis, neutrophilia, and monocytosis, were consistent with changes associated with inflammation and correlated with the tail swelling and tail necrosis observed for rats in the 80 mg/kg/day AL dose group. Clinical pathological changes observed on Day 15 for female rats given 150 mg/kg/day of AS for 7 consecutive days were also consistent with an effect of AS on hematopoiesis. In contrast, a possible hematopoietic effect was not observed following 7-day im administration of AE to rats; clinical pathological changes observed for rats in the AE dose group were limited to minimal increases in group mean reticulocyte percents and counts.

No evidence was obtained for the presence of histopathological lesions in the hindbrains of rats given doses of ≤150 mg/kg/day of AS for 7 consecutive days. In addition, no evidence was obtained for the occurrence of histopathological lesions in the hindbrains of rats given ≤37.5 mg/kg/day of AL for 7 consecutive days; due to the problematic dosing, no conclusions could be made regarding the possible neuropathy produced by multiple dose administration of higher doses of AL. Histopathological lesions were observed, however, for rats given daily im doses of 25 mg/kg/day of AE for 7 consecutive days.

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Table 1

Levels of Radioactivity in Whole Blood following IV Administration of [^{14}C]Artelinic Acid to Dogs

Sample Time (hr)	AM2249 μgeq/mL	AM2250 μgeq/mL	AF2253 μgeq/mL	AF2255 μgeq/mL	Mean μgeq/mL	S.D.
0.083	19.1	22.9	21.2	18.6	20.5	2.0
0.25	14.1	16.5	15.9	14.4	15.2	1.1
0.5	9.4	11.5	10.8	9.8	10.4	0.9
1	4.9	6.7	5.7	5.5	5.7	0.8
2	2.6	3.5	2.6	2.5	2.8	0.5
4	2.9	3.6	1.6	1.9	2.5	0.9
8	1.8	1.7	1.2	1.9	1.6	0.3
12	1.6	2.5	1.2	1.3	1.7	0.6
24	0.76	1.3	0.93	0.79	0.95	0.3
48	0.31	0.43	0.41	0.33	0.37	0.1
72	0.22	0.29	0.29	0.23	0.26	0.04
96	0.18	0.25	0.22	0.21	0.21	0.03
120	0.15	0.24	0.21	0.18	0.19	0.04
144	0.15	0.21	0.17	0.17	0.18	0.03
192	0.14	0.18	0.16	0.15	0.16	0.02

Table 2

Levels of Radioactivity in Plasma following IV Administration of [^{14}C]Artelinic Acid to Dogs

Sample Time (hr)	AM2249 μgeq/mL	AM2250 μgeq/mL	AF2253 μgeq/mL	AF2255 μgeq/mL	Mean μgeq/mL	S.D.
0.083	30.3	36.4	32.3	31.7	32.7	2.6
0.25	22.0	25.3	24.7	23.5	23.9	1.4
0.5	14.8	17.6	16.9	15.3	16.2	1.3
1	7.7	10.1	8.6	8.5	8.7	1.0
2	4.1	5.3	4.0	4.0	4.4	0.6
4	4.6	5.4	2.4	2.8	3.8	1.4
8	3.1	3.6	2.0	3.4	3.0	0.7
12	2.9	3.9	1.8	2.7	2.8	0.9
24	1.2	2.0	1.3	1.2	1.4	0.4
48	0.41	0.52	0.51	0.43	0.47	0.1
72	0.27	0.33	0.34	0.29	0.31	0.03
96	0.21	0.30	0.30	0.26	0.27	0.04
120	0.18	0.25	0.25	0.20	0.22	0.03
144	0.16	0.22	0.20	0.18	0.19	0.02
192	0.12	0.17	0.18	0.15	0.15	0.03

Table 3

Concentrations of Unchanged Artelinic Acid in Plasma following IV Administration of [^{14}C]Artelinic Acid to Dogs

Sample Time (hr)	AM2249 μg/mL	AM2250 μg/mL	AF2253 μg/mL	AF2255 μg/mL	Mean μg/mL	S.D.
0.083	16.4	24.9	25.3	18.2	21.2	4.6
0.25	9.2	11.9	11.6	9.4	10.5	1.4
0.5	7.4	8.1	7.5	4.7	6.9	1.5
1	2.4	2.6	2.0	1.0	2.0	0.7
2	3.1	0.5	0.1	0.2	1.0	1.4
4	0.5	ND	ND	ND	NA	NA
8	ND	ND	ND	ND	NA	NA
12	ND	ND	ND	ND	NA	NA
24	ND	ND	ND	ND	NA	NA
48	ND	ND	ND	ND	NA	NA
72	ND	ND	ND	ND	NA	NA
96	ND	ND	ND	ND	NA	NA
120	ND	ND	ND	ND	NA	NA
144	ND	ND	ND	ND	NA	NA
192	ND	ND	ND	ND	NA	NA

ND = below the limit of quantification (1 ng/mL)

NA = Not applicable

Table 4

Parameters Calculated from Pharmacokinetic Analysis
of Concentrations of **Radioactivity in Whole Blood** following
IV Administration of [^{14}C]Artelinic Acid to Dogs

Compartmental Analysis						
Parameter	AM2249	AM2250	AF2253	AF2255	Mean	S.D.
C_0 (µg equiv/mL)[a]	23.0	25.3	23.0	21.6	23.2	1.5
$t_{½\alpha}$ (hours)[b]	0.295	0.365	0.427	0.366	0.363	0.05
$t_{½\beta}$ (hours)[c]	10.5	12.2	17.6	11.7	13.0	3.1
$t_{½\gamma}$ (hours)[d]	288	284	376	284	308	45.4
AUC_{inf} (µg equiv•hr/mL)[e]	141	187	174	147	162	21.9
Clearance (mL/hr/kg)[f]	68.3	51.5	55.4	65.7	60.2	8.1
Vd_{ss} (mL/kg)[g]	18241	13907	21375	18447	17993	3077

Non-Compartmental Analysis						
Parameter	AM2249	AM2250	AF2253	AF2255	Mean	S.D.
C_0 (µg equiv/mL)[a]	22.2	27.0	24.5	21.1	23.7	2.6
$t_{½}$ (hours)[d]	231	182	165	202	195	28.4
AUC_{inf} (µg equiv•hr/mL)[e]	134	171	128	131	141	20.1

a Concentration of radioactivity in whole blood at time 0
b Half-life of the distribution phase
c Half-life of the initial elimination phase
d Half-life of the terminal elimination phase
e Area under the concentration versus time curve calculated from 0 to infinity
f Total body clearance
g Volume of distribution at steady state

Table 5

Pharmacokinetic Parameters Calculated from Concentrations of **Radioactivity in Plasma** following **IV** Administration of [^{14}C]Artelinic Acid to Dogs

Compartmental Analysis						
Parameter	AM2249	AM2250	AF2253	AF2255	Mean	S.D.
C_0 (µg equiv/mL)[a]	36.9	42.4	36.1	36.9	38.1	2.9
$t_{½\alpha}$ (hours)[b]	0.28	0.29	0.41	0.33	0.33	0.06
$t_{½\beta}$ (hours)[c]	10.0	10.7	14.1	11.3	11.5	1.8
$t_{½\gamma}$ (hours)[d]	130	171	183	170	164	23.1
AUC_{inf} (µg equiv•hr/mL)[e]	147	205	168	159	170	25.0
Clearance (mL/hr/kg)[f]	65.7	47.1	57.3	60.5	57.7	7.8
Vd_{ss} (mL/kg)[g]	5805	5753	9027	7907	7123	1618

Non-Compartmental Analysis						
Parameter	AM2249	AM2250	AF2253	AF2255	Mean	S.D.
C_0 (µg equiv/mL)[a]	35.5	43.6	36.9	36.8	38.2	3.7
$t_{½}$ (hours)[d]	112	123	134	132	125	10.0
AUC_{inf} (µg equiv•hr/mL)[e]	152	207	161	160	170	25.0

[a] Concentration of radioactivity in plasma at time 0
[b] Half-life of the distribution phase
[c] Half-life of the initial elimination phase
[d] Half-life of the terminal elimination phase
[e] Area under the concentration versus time curve calculated from 0 to infinity
[f] Total body clearance
[g] Volume of distribution at steady state

Table 6

Pharmacokinetic Parameters Calculated from **Compartmental Analysis** of Concentrations of **Unchanged Artelinic Acid** in Plasma following **IV or PO** Administration of [^{14}C]Artelinic Acid to Dogs

IV Dose						
Parameter	AM2249	AM2250	AF2253	AF2255	Mean	S.D.
$t_{½\alpha}$ (hours)[a]	0.16	0.08	0.02	0.18	0.11	0.07
$t_{½\beta}$ (hours)[b]	1.3	0.41	0.26	0.59	0.64	0.46
AUC_{inf} (µg equiv•hr/mL)[c]	12.5	11.7	11.7	7.4	10.8	2.3
Clearance (mL/hr/kg)[d]	765	821	820	1290	924	245
Vd_{ss} (mL/kg)[e]	1021	398	257	505	545	333

PO Dose						
Parameter	BM2251	BM2252	BF2254	BF2256	Mean	S.D.
C_{max} (µg equiv/mL)[f]	2.8	3.3	3.4	4.2	3.4	0.6
T_{max} (hours)[g]	0.7	0.6	0.7	0.6	0.7	0.06
$t_{½}$ (hours)[b]	1.8	0.42	0.31	0.39	0.73	0.71
k_a (hours^{-1})[h]	3.2	1.6	1.0	1.7	1.9	0.95
AUC_{inf} (µg equiv•hr/mL)[c]	9.5	5.4	6.5	6.5	7.0	1.7
F (%)[i]	--	--	--	--	67	--

a Half-life of the initial elimination phase
b Half-life of the terminal elimination phase
c Area under the concentration versus time curve calculated from 0 to infinity
d Total body clearance
e Volume of distribution at steady state
f Maximum concentration of radioactivity in plasma
g Time of maximum concentration of radioactivity in plasma
h Absorption rate constant
i Bioavailability; calculated, using mean AUC_{inf} values, from the following equation:
$F = (AUC_{infPO} \times Dose_{IV} / AUC_{infIV} \times Dose_{PO}) \times 100$

Table 7

Urinary and Fecal Elimination of Radioactivity through 192 Hours following IV Administration of [^{14}C]Artelinic Acid to Dogs

Sample Time (hr)	Animal ID	Urine		Rinse		Feces		Total
		μgeq	% Dose	μgeq	% Dose	μgeq	% Dose	% Dose
12	AM2249	12261	12.9	7431	7.8	7443	7.8	28.4
24		7397	7.8	2248	2.4	36040	37.8	47.9
48		1654	1.7	1095	1.1	8360	8.8	11.6
72		401	0.4	75	0.1	1539	1.6	2.1
96		213	0.2	91	0.1	965	1.0	1.3
120		107	0.1	36	0.04	163	0.2	0.3
144		301	0.3	63	0.1	223	0.2	0.6
168		51	0.1	46	0.05	75	0.1	0.2
192		29	0.03	25	0.03	40	0.04	0.1
Total		22414	23.5	11111	11.6	54848	57.5	92.6
12	AM2250	35818	33.5	6195	5.8	19714	18.4	57.7
24		8302	7.8	1122	1.0	11980	11.2	20.0
48		2233	2.1	503	0.5	10074	9.4	12.0
72		412	0.4	77	0.1	1756	1.6	2.1
96		193	0.2	67	0.1	438	0.4	0.7
120		98	0.1	39	0.04	359	0.3	0.5
144		31	0.03	16	0.02	109	0.1	0.1
168		54	0.1	34	0.03	87	0.1	0.2
192		34	0.03	62	0.1	52	0.05	0.1
Total		47174	44.1	8116	7.6	44568	41.7	93.4

Table 7 (Continued)

Urinary and Fecal Elimination of Radioactivity through 192 Hours following IV Administration of [^{14}C]Artelinic Acid to Dogs

Sample Time (hr)	Animal ID	Urine		Rinse		Feces		Total
		μgeq	% Dose	μgeq	% Dose	μgeq	% Dose	% Dose
12	AF2253	16787	20.7	4979	6.2	123	0.2	27.0
24		11944	14.8	826	1.0	24630	30.4	46.2
48		3214	4.0	335	0.4	7341	9.1	13.5
72		966	1.2	93	0.1	2252	2.8	4.1
96		408	0.5	65	0.1	1864	2.3	2.9
120		102	0.1	49	0.1	180	0.2	0.4
144		52	0.1	20	0.02	109	0.1	0.2
168		31	0.04	15	0.02	49	0.1	0.1
192		22	0.03	0[a]	0.0[a]	20	0.02	0.1
Total		33527	41.4	6383	7.9	36568	45.2	94.5
12	AF2255	26523	32.0	3629	4.4	1671	2.0	38.4
24		4246	5.1	2108	2.5	23516	28.4	36.0
48		1561	1.9	586	0.7	9899	11.9	14.5
72		254	0.3	78	0.1	991	1.2	1.6
96		115	0.1	60	0.1	385	0.5	0.7
120		66	0.1	30	0.04	167	0.2	0.3
144		49	0.1	16	0.02	370	0.4	0.5
168		38	0.05	26	0.03	44	0.1	0.1
192		22	0.03	15	0.02	27	0.03	0.1
Total		32873	39.7	6548	7.9	37071	44.7	92.3

[a] Sample was mis-identified and lost during processing for analysis

Table 8

Summary of the Urinary and Fecal Elimination of Radioactivity following IV or PO Administration of [^{14}C]Artelinic Acid to Dogs

Animal ID	Dose Route	Percent of Dose[a]			
		Urine	Rinse	Feces	Total
AM2249	IV	23.5	11.6	57.5	92.6
AM2250	IV	44.1	7.6	41.7	93.4
AF2253	IV	41.4	7.9	45.2	94.5
AF2255	IV	39.7	7.9	44.7	92.3
Mean		37.2	8.8	47.3	93.2
S.D.		9.3	1.9	7.0	1.0
BM2251	PO	24.8	8.4	60.1	93.4
BM2252	PO	25.9	11.5	53.4	90.8
BF2254	PO	18.5	17.4	57.2	93.1
BF2256	PO	32.7	2.3	59.2	94.3
Mean		25.5	9.9	57.5	92.9
S.D.		5.8	6.3	3.0	1.5

[a]Values were obtained from Tables 11 and 24

Table 9

Metabolite Profiles of Urine Collected 0-12 Hours following IV or PO Administration of [^{14}C]Artelinic Acid to Dogs

Animal ID	Time (hr)	Dose Route	% Radioactivity Eluting at a Retention Time of (minutes)[a]							
			15.5	43.3	46	49.3	58.5	59.7	61.9	76.3
AM2249	0-12	IV	7.7	53.3	6.7	16.1	4.4	2.6	1.6	3.6
AM2250	0-12	IV	7.7	48.8	11.5	15.5	2.6	2.2	3.4	3.6
AF2253	0-12	IV	7.4	43.1	9.2	16.8	1.5	1.7	0.9	1.5
AF2255	0-12	IV	6.6	45.2	6.2	16.2	2.4	3.0	4.2	3.8
BM2251	0-12	PO	7.1	39.4	4.7	17.3	8.9	2.1	6.1	3.6
BM2252	0-12	PO	5.6	50.9	7.1	12.9	3.5	3.2	6.2	8.2
BF2254	0-12	PO	5.9	54.0	6.8	16.4	3.9	ND[b]	ND	7.5
BF2256	0-12	PO	8.5	68.4	ND	18.1	2.2	ND	ND	ND

[a]Under the conditions of analysis used, artelinic acid had a retention time of approximately 85 minutes.

[b]ND = A peak was not detected at the indicated retention time

Table 10

Summary of the Urinary Excretion of Radioactivity 0-12 Hours following IV or PO Administration of [^{14}C]Artelinic Acid to Dogs

Animal ID	Time (hr)	Dose Route	%Dose/peak at a Retention Time of (minutes)							
			15.5	43.3	46	49.3	58.5	59.7	61.9	76.3
AM2249	0-12	IV	1.0	6.9	0.9	2.1	0.6	0.3	0.2	0.5
AM2250	0-12	IV	2.6	16.3	3.9	5.2	0.9	0.7	1.1	1.2
AF2253	0-12	IV	1.5	8.9	1.9	3.5	0.3	0.4	0.2	0.3
AF2255	0-12	IV	2.1	2.1	2.0	5.2	0.8	1.0	1.0	1.2
BM2251	0-12	PO	1.1	7.8	1.1	2.8	0.4	0.5	1.1	0.6
BM2252	0-12	PO	0.5	4.5	0.6	1.1	0.3	0.3	0.6	0.7
BF2254	0-12	PO	0.1	0.8	0.1	0.2	0.1	ND	ND	0.1
BF2256	0-12	PO	1.5	11.7	ND	3.1	0.4	ND	ND	ND

Table 11

Metabolite Profiles of Urine Collected 12-24 Hours following IV or PO Administration of [^{14}C]Artelinic Acid to Dogs

Animal ID	Time (hr)	Dose Route	% Radioactivity Eluting at a Retention Time of (minutes)[a]						
			15.5	37.5	43.3	46	49.3	58.5	70.2
AM2249	12-24	IV	9.5	2.2	26.0	6.3	13.4	22.5	3.8
AM2250	12-24	IV	7.6	5.6	32.0	8.2	8.7	25.4	7.9
AF2253	12-24	IV	11.6	4.6	40.1	12.9	22.6	8.1	ND[b]
AF2255	12-24	IV	7.9	2.6	31.0	3.9	12.7	20.4	ND
BM2251	12-24	PO	7.3	3.8	30.9	5.8	14.4	17.7	4.9
BM2252	12-24	PO	7.5	ND	38.8	6.5	12.7	16.8	ND
BF2254	12-24	PO	6.9	2.6	33.8	10.2	23.6	10.2	ND
BF2256	12-24	PO	6.7	3.1	40.0	7.8	13.6	22.1	ND

[a]Under the conditions of analysis used, artelinic acid had a retention time of approximately 85 minutes.

[b]ND = A peak was not detected at the indicated retention time

Table 12

Summary of the Urinary Excretion of Radioactivity 12-24 Hours following IV or PO Administration of [^{14}C]Artelinic Acid to Dogs

Animal ID	Time (hr)	Dose Route	%Dose/peak at a Retention Time of (minutes)						
			15.5	37.5	43.3	46	49.3	58.5	70.2
AM2249	12-24	IV	0.74	0.17	2.0	0.5	1.0	1.7	0.3
AM2250	12-24	IV	0.6	0.4	2.5	0.6	0.7	2.0	0.6
AF2253	12-24	IV	1.7	0.7	5.9	1.9	3.3	1.2	ND
AF2255	12-24	IV	0.4	0.1	0.4	0.2	0.7	1.0	ND
BM2251	12-24	PO	0.2	0.1	1.0	0.2	0.5	0.6	0.2
BM2252	12-24	PO	0.9	ND	4.6	0.8	1.5	2.0	ND
BF2254	12-24	PO	0.9	0.3	4.4	1.3	3.1	1.3	ND
BF2256	12-24	PO	0.8	0.4	4.9	ND	1.7	2.7	ND

Table 13

Metabolite Profiles of Feces Collected 0-12 or 12-24 Hours following IV or PO Administration of [^{14}C]Artelinic Acid to Dogs

Animal ID	Time (hr)	Dose Route	% Radioactivity Eluting at a Retention Time of (minutes)[a]		
			60	76	79
AM2249	12-24	IV	21.1	28.7	50.2
AM2250	0-12	IV	7.2	57.4	35.4
AF2253	12-24	IV	18.3	43.3	38.4
AF2255	12-24	IV	11.8	40.5	47.7
BM2251	12-24	PO	9.7	34.1	50.5
BM2252	12-24	PO	7.0	42.6	45.0
BF2254	12-24	PO	13	29.1	48.1
BF2256	12-24	PO	ND[b]	26.2	51.9

[a]Under the conditions of analysis used, artelinic acid had a retention time of approximately 85 minutes

[b]ND = a peak was not detected at the indicated retention time

Table 14

Summary of the Fecal Elimination of Radioactivity 0-12 or 12-24 Hours After IV or PO Administration of [^{14}C]Artelinic Acid to Dogs

Animal ID	Time (hr)	Dose Route	% Dose/Peak at Retent. Time of (min):		
			60	76	79
AM2249	12-24	IV	5.5	7.5	13.0
AM2250	0-12	IV	0.8	6.7	4.1
AF2253	12-24	IV	3.7	8.8	7.8
AF2255	12-24	IV	2.1	7.3	8.6
BM2251	12-24	PO	2.2	7.8	11.5
BM2252	12-24	PO	1.3	7.7	8.1
BF2254	12-24	PO	3.1	7.0	11.6
BF2256	12-24	PO	ND	6.6	13.0

Table 15

Levels of Radioactivity in Whole Blood following PO Administration of [^{14}C]Artelinic Acid to Dogs

Sample Time (hr)	BM2251 μgeq/mL	BM2252 μgeq/mL	BF2254 μgeq/mL	BF2256 μgeq/mL	Mean μgeq/mL	S.D.
0.5	5.1	7.2	7.5	6.7	6.6	1.1
1	4.2	4.4	5.4	4.9	4.7	0.6
2	2.7	2.9	3.5	2.5	2.9	0.4
4	2.8	1.8	2.2	1.7	2.2	0.5
8	1.7	1.2	1.8	1.6	1.6	0.3
12	1.6	1.7	1.4	1.0	1.4	0.3
24	0.73	1.5	0.95	0.57	0.93	0.4
48	0.31	0.40	0.39	0.30	0.35	0.1
72	0.24	0.24	0.34	0.22	0.26	0.1
96	0.22	0.19	0.26	0.17	0.21	0.04
120	0.19	0.17	0.22	0.17	0.19	0.02
144	0.15	0.16	0.19	0.14	0.16	0.02
192	0.15	0.13	0.18	0.13	0.15	0.03

Table 16

Levels of Radioactivity in Plasma following PO Administration of [^{14}C]Artelinic Acid to Dogs

Sample Time (hr)	BM2251 μgeq/mL	BM2252 μgeq/mL	BF2254 μgeq/mL	BF2256 μgeq/mL	Mean μgeq/mL	S.D.
0.5	8.5	11.6	12.3	10.8	10.8	1.7
1	7.0	6.5	9.2	7.7	7.6	1.2
2	4.5	4.0	5.9	3.9	4.6	0.9
4	5.0	2.7	3.7	2.6	3.5	1.1
8	3.5	2.5	3.4	3.0	3.1	0.5
12	2.5	2.8	2.5	1.7	2.4	0.5
24	1.1	2.1	1.4	0.78	1.4	0.6
48	0.36	0.50	0.54	0.38	0.44	0.1
72	0.27	0.27	0.41	0.25	0.30	0.1
96	0.23	0.22	0.33	0.21	0.25	0.1
120	0.18	0.18	0.26	0.19	0.20	0.04
144	0.16	0.17	0.22	0.16	0.18	0.03
192	0.14	0.14	0.20	0.11	0.15	0.04

Table 17

Concentrations of Unchanged Artelinic Acid in Plasma following PO Administration of [^{14}C]Artelinic Acid to Dogs

Sample Time (hr)	BM2251 μg/mL	BM2252 μg/mL	BF2254 μg/mL	BF2256 μg/mL	Mean μg/mL	S.D.
0.5	2.5	3.5	3.1	3.7	3.2	0.5
1	3.3	2.4	3.0	4.2	3.2	0.8
2	1.7	1.5	2.8	1.3	1.8	0.7
4	0.9	0.1	0.2	0.1	0.3	0.4
8	ND[a]	ND	0.06[b]	ND	NA[c]	NA
12	ND	ND	ND	ND	NA	NA
24	ND	ND	ND	ND	NA	NA
48	ND	ND	ND	ND	NA	NA
72	ND	ND	ND	ND	NA	NA
96	ND	ND	ND	ND	NA	NA
120	ND	ND	ND	ND	NA	NA
144	ND	ND	ND	ND	NA	NA
192	ND	ND	ND	ND	NA	NA

[a] ND = below the limit of quantification (1 ng/mL)

[b] Concentration in sample was below that for the lowest standard

[c] NA = not applicable

Table 18

Pharmacokinetic Parameters Calculated from **Non-Compartmental Analysis** of Concentrations of **Radioactivity in Whole Blood** and **Plasma** following **PO** Administration of [^{14}C]Artelinic Acid to Dogs

Whole Blood						
Parameter	BM2251	BM2252	BF2254	BF2256	Mean	S.D.
C_{max} (µg equiv/mL)[a]	5.1	7.2	7.5	6.7	6.6	1.1
T_{max} (hours)[b]	0.5	0.5	0.5	0.5	0.5	0.0
$t_{½}$ (hours)[c]	173	156	159	185	168	13.4
AUC_{inf} (µg equiv•hr/mL)[d]	118	124	134	103	120	13.0
F (%)[e]	--	--	--	--	85	--

Plasma						
Parameter	BM2251	BM2252	BF2254	BF2256	Mean	S.D.
C_{max} (µg equiv/mL)[a]	8.5	11.6	12.3	10.8	10.8	1.7
T_{max} (hours)[b]	0.5	0.5	0.5	0.5	0.5	0.0
$t_{½}$ (hours)[c]	139	144	133	99	129	20.3
AUC_{inf} (µg equiv•hr/mL)[d]	146	161	178	114	150	27.2
F (%)[e]	--	--	--	--	88	--

[a] Maximum concentration of radioactivity in whole blood
[b] Time of the maximum concentration of radioactivity in whole blood
[c] Half-life of the terminal elimination phase
[d] Area under the concentration versus time curve calculated from 0 to infinity
[e] Bioavailability; calculated, using mean AUC_{inf} values, from the following equation:
$F = (AUC_{infPO} \times Dose_{IV} / AUC_{infIV} \times Dose_{PO}) \times 100$

Table 19

Urinary and Fecal Elimination of Radioactivity through 192 Hours
following PO Administration of [^{14}C]Artelinic Acid to Dogs

Sample Time (hr)	Animal ID	Urine		Rinse		Feces		Total % Dose
		μg/eq	% Dose	μg/eq	% Dose	μg/eq	% Dose	
12	BM2251	12738	17.3	2441	3.3	3223	4.4	25.0
24		2495	3.4	2378	3.2	26247	35.7	42.3
48		2197	3.0	505	0.7	12971	17.6	21.3
72		594	0.8	579	0.8	703	1.0	2.6
96		58	0.1	121	0.2	224	0.3	0.5
120		66	0.1	53	0.1	296	0.4	0.6
144		42	0.1	25	0.03	434	0.6	0.7
168		24	0.03	71	0.10	67	0.09	0.2
192		30	0.04	19	0.03	21	0.03	0.1
Total		18244	24.8	6193	8.4	44186	60.1	93.4
12	BM2252	9103	8.9	8796	8.6	144	0.1	17.6
24		12085	11.8	1449	1.4	27199	26.5	39.7
48		3616	3.5	1018	1.0	20768	20.3	24.8
72		814	0.8	243	0.2	3863	3.8	4.8
96		511	0.5	113	0.1	1751	1.7	2.3
120		214	0.2	88	0.1	609	0.6	0.9
144		84	0.1	21	0.02	239	0.2	0.3
168		79	0.1	39	0.04	158	0.2	0.3
192		41	0.04	46	0.04	31	0.03	0.1
Total		26548	25.9	11815	11.5	54762	53.4	90.8

Table 19 (Continued)

Urinary and Fecal Elimination of Radioactivity through 192 Hours
following PO Administration of [^{14}C]Artelinic Acid to Dogs

Sample Time (hr)	Animal ID	Urine		Rinse		Feces		Total
		μg/eq	% Dose	μg/eq	% Dose	μg/eq	% Dose	% Dose
12	BF2254	1372	1.4	11252	11.5	580	0.6	13.5
24		12865	13.1	3846	3.9	36242	37.0	54.0
48		2062	2.1	1133	1.2	14549	14.8	18.1
72		809	0.8	303	0.3	1240	1.3	2.4
96		596	0.6	254	0.3	2073	2.1	3.0
120		192	0.2	98	0.1	891	0.9	1.2
144		89	0.1	54	0.1	295	0.3	0.4
168		62	0.1	95	0.1	135	0.1	0.3
192		59	0.1	48	0.05	59	0.1	0.2
Total		18106	18.5	17083	17.4	56065	57.2	93.1
12	BF2256	11367	17.2	1140	1.7	1146	1.7	20.6
24		8130	12.3	269	0.4	29119	44.0	56.6
48		1361	2.1	70	0.1	6409	9.7	11.8
72		454	0.7	31	0.05	1498	2.3	3.0
96		178	0.3	20	0.03	479	0.7	1.0
120		92	0.1	9	0.01	281	0.4	0.6
144		28	0.04	NS[a]	0.0	254	0.4	0.4
168		28	0.04	NS	0.0	37	0.1	0.1
192		20	0.03	NS	0.0	17	0.03	0.1
Total		21657	32.7	1540	2.3	39240	59.2	94.3

[a] NS = not significant; radioactivity in portion of sample assayed was less than twice background

Table 20

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Dose Formulation/Homogeneity Analysis: Artelinic Acid

Dose Level (mg/kg/day)	Analysis Dates	Concentration (mg/mL)									
		Theoretical	Actual							Percent (%) RSD	Percent (%) Theoretical [a]
			Sample No.						Sample Average		
			1	2	3	4	5	6			
10	8/24/99[b]	2.00	1.58	1.51	1.56	NA	NA	NA	1.55	2.4	77
20	8/24/99[b]	4.00	3.36	3.05	3.01	NA	NA	NA	3.14	6.0	79
40	8/24/99[b]	8.00	7.91	7.55	7.75	NA	NA	NA	7.74	2.4	97
80	8/24/99[b]	16.0	15.0	11.3	16.0	NA	NA	NA	14.1	18	88
320	8/24/99[b]	64.0	48.3	70.5	67.6	NA	NA	NA	62.1	19	97
10	8/25/99	2.00	1.61	1.57	1.60	1.54	1.52	1.56	1.57	2.2	78
20	8/25/99	4.00	3.44	3.38	3.21	3.55	3.33	3.47	3.40	3.4	85
40	8/25/99	8.00	7.23	6.94	7.05	6.43	6.79	6.45	6.81	4.8	85
80	8/25/99	16.0	15.1	13.8	14.9	15.1	14.9	13.7	14.6	4.6	91
320	8/25/99	64.0	63.3	57.0	59.0	58.2	62.3	62.7	60.4	4.4	94
10	8/31/99[b]	2.00	1.50	1.44	1.48	NA	NA	NA	1.48	2.1	74
20	8/31/99[b]	4.00	3.43	3.45	3.54	NA	NA	NA	3.47	1.6	87
40	8/31/99[b]	8.00	6.18	6.52	6.78	NA	NA	NA	6.50	4.6	81
80	8/31/99[b]	16.0	13.9	14.0	14.7	NA	NA	NA	14.2	3.1	89
320	8/31/99[b]	64.0	53.5	54.4	54.8	NA	NA	NA	54.2	1.2	85
10	9/10/99[c]	2.00	1.54	1.45	1.42	NA	NA	NA	1.47	4.4	74
20	9/10/99[c]	4.00	3.69	3.97	3.97	NA	NA	NA	3.88	4.2	97
40	9/10/99[c]	8.00	5.55	6.41	6.38	NA	NA	NA	6.11	8.0	76
80	9/10/99[c]	16.0	15.5	15.5	14.2	NA	NA	NA	15.1	4.9	94
320	9/10/99[c]	64.0	63.4	NR	63.1	NA	NA	NA	63.2	0.31	99

NA = not applicable
NR = not recorded; outlier
RSD = relative standard deviation
Calculated values reported are based upon nontruncated numbers and may not be reproducible based upon the rounded values that appear in the table.
[a] Percent Theoretical = $\frac{\text{Actual Concentration}}{\text{Theoretical Concentration}} \times 100$
[b] Homogeneity samples taken from the top (Sample 1), middle (Sample 2), and bottom (Sample 3) of the formulation.
[c] Post dose formulation analysis; results reported are from formulations mixed on 8/30/99 and analyzed (predose) on 8/31/99.

Table 21

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Summary Clinical Observations: Males

	Group V 0 mg/kg/day	Group A 10 mg/kg/day Artelinic Acid	Group B 20 mg/kg/day Artelinic Acid	Group C 40 mg/kg/day Artelinic Acid	Group D 80 mg/kg/day Artelinic Acid	Group E 320 mg/kg/day Artelinic Acid	Group F 12.5 mg/kg/day Arteether
Unremarkable							
Number of Observations	34	66	68	66	66	10	34
Number of Animals	2	4	4	4	4	2	2
Days from - to	1 21	1 21	1 21	1 21	1 21	2 6	1 21
Dehydrated							
Number of Observations	.	.	.	.	.	2	.
Number of Animals	.	.	.	.	.	2	.
Days from - to	.	.	.	.	.	16 16	.
Discharge							
Number of Observations	.	.	.	.	.	4	.
Number of Animals	.	.	.	.	.	2	.
Days from - to	.	.	.	.	.	15 16	.
Emaciated							
Number of Observations	.	.	.	.	.	30	.
Number of Animals	.	.	.	.	.	2	.
Days from - to	.	.	.	.	.	7 21	.
Hypoactive							
Number of Observations	.	.	.	.	.	1	.
Number of Animals	.	.	.	.	.	1	.
Days from - to	.	.	.	.	.	18 18	.
Tics							
Number of Observations	.	.	.	.	.	1	.
Number of Animals	.	.	.	.	.	1	.
Days from - to	.	.	.	.	.	18 18	.

Table 21 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Summary Clinical Observations: Females

	Group V 0 mg/kg/day	Group A 10 mg/kg/day Artelinic Acid	Group B 20 mg/kg/day Artelinic Acid	Group C 40 mg/kg/day Artelinic Acid	Group D 80 mg/kg/day Artelinic Acid	Group E 320 mg/kg/day Artelinic Acid	Group F 12.5 mg/kg/day Arteether
Unremarkable							
Number of Observations	32	66	66	66	66	14	34
Number of Animals	2	4	4	4	4	2	2
Days from - to	2 21	1 21	1 21	1 21	1 21	1 7	1 21
Discharge							
Number of Observations	.	.	.	.	.	1	.
Number of Animals	.	.	.	.	.	1	.
Days from - to	.	.	.	.	.	9 9	.
Emaciated							
Number of Observations	.	.	.	.	.	6	.
Number of Animals	.	.	.	.	.	2	.
Days from - to	.	.	.	.	.	8 11	.

Table 22

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Summary Body Weights (grams): Males

Group Sex		Day numbers relative to Start Date												
		-2	-1	1	2	3	4	5	6	7	8	9	10	11
VM	Mean	.	256.40	265.90	271.75	278.30	286.75	291.70	297.85	305.90	310.85	314.50	321.90	328.95
	S.D.	.	.	.	.	.	.	.	.	.	.	.	.	.
	N	0	2	2	2	2	2	2	2	2	2	2	2	2
AM	Mean	249.90	264.00	261.60	262.85	269.85	277.18	283.90	288.70	295.30	300.43	305.93	311.85	315.58
	S.D.	.	.	6.48	14.44	11.94	9.99	6.77	6.03	6.58	4.89	5.81	7.84	6.06
	N	2	2	4	4	4	4	4	4	4	4	4	4	4
BM	Mean	256.80	258.93	267.75	271.35	279.18	286.35	292.80	299.10	309.68	314.45	320.43	330.90	335.18
	S.D.	.	10.64	12.88	11.34	14.62	14.42	13.44	11.05	15.37	14.09	15.93	16.92	16.75
	N	2	4	4	4	4	4	4	4	4	4	4	4	4
CM	Mean	265.95	251.50	271.70	274.80	281.25	286.03	289.85	294.30	300.45	307.63	309.43	314.63	320.20
	S.D.	.	.	12.10	12.27	14.12	9.65	12.35	11.48	14.91	9.84	13.28	14.77	15.86
	N	2	2	4	4	4	4	4	4	4	4	4	4	4
DM	Mean	258.15	263.83	274.05	273.38	278.85	283.00	284.25	288.83	296.80	302.30	295.93	303.50	308.25
	S.D.	.	6.34	5.73	8.71	9.08	7.18	7.66	8.40	10.34	9.78	15.48	10.55	12.03
	N	2	4	4	4	4	4	4	4	4	4	4	4	4
EM	Mean	253.10	266.30	272.90	257.20	251.25	244.20	237.20	233.45	227.25	221.75	218.05	210.20	204.80
	S.D.	.	.	.	.	.	.	.	.	.	.	.	.	.
	N	2	2	2	2	2	2	2	2	2	2	2	2	2
FM	Mean	.	260.35	270.95	278.75	283.70	289.45	297.05	300.25	290.95	312.75	316.95	316.80	323.65
	S.D.	.	.	.	.	.	.	.	.	.	.	.	.	.
	N	0	2	2	2	2	2	2	2	2	2	2	2	2

(.) = Not applicable
[a] All animals in group dead prior to scheduled collection period.
Group V - 0 mg/kg/day
Group A - 10 mg/kg/day Artelinic Acid
Group B - 20 mg/kg/day Artelinic Acid
Group C - 40 mg/kg/day Artelinic Acid
Group D - 80 mg/kg/day Artelinic Acid
Group E - 320 mg/kg/day Artelinic Acid
Group F - 12.5 mg/kg/day Arteether

Table 22 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Summary Body Weights (grams): Males

Group Sex		Day numbers relative to Start Date			
		12	13	14	21
VM	Mean	333.20	337.15	345.95	357.85
	S.D.	.	.	.	.
	N	2	2	2	2
AM	Mean	319.88	325.90	328.33	337.18
	S.D.	10.14	9.88	8.19	8.32
	N	4	4	4	4
BM	Mean	332.10	348.38	350.63	366.30
	S.D.	19.41	17.42	14.45	17.27
	N	4	4	4	4
CM	Mean	326.65	332.48	337.25	352.78
	S.D.	18.17	17.96	18.86	22.12
	N	4	4	4	4
DM	Mean	310.25	317.85	325.40	345.00
	S.D.	11.84	11.19	16.80	16.04
	N	4	4	4	4
EM	Mean	199.90	198.40	197.85	195.30
	S.D.	.	.	.	.
	N	2	2	2	2
FM	Mean	331.35	332.10	333.50	344.30
	S.D.	.	.	.	.
	N	2	2	2	2

(.) = Not applicable
[a] All animals in group dead prior to scheduled collection period.
Group V - 0 mg/kg/day
Group A - 10 mg/kg/day Artelinic Acid
Group B - 20 mg/kg/day Artelinic Acid
Group C - 40 mg/kg/day Artelinic Acid
Group D - 80 mg/kg/day Artelinic Acid
Group E - 320 mg/kg/day Artelinic Acid
Group F - 12.5 mg/kg/day Arteether

Table 22 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Summary Body Weights (grams): Females

Group Sex		Day numbers relative to Start Date												
		-2	-1	1	2	3	4	5	6	7	8	9	10	11
VF	Mean	205.60	.	216.20	215.35	220.50	225.75	228.70	227.35	229.10	237.50	240.85	239.95	241.65
	S.D.	.	.	.	.	.	.	.	.	.	.	.	.	.
	N	2	0	2	2	2	2	2	2	2	2	2	2	2
AF	Mean	209.15	210.60	213.70	212.38	215.13	219.98	218.98	218.33	223.58	227.95	227.88	227.78	231.18
	S.D.	.	9.48	6.36	9.26	10.86	9.02	6.70	9.63	11.40	9.54	6.51	9.25	10.15
	N	2	4	4	4	4	4	4	4	4	4	4	4	4
BF	Mean	203.50	211.93	213.18	214.03	215.23	217.83	217.68	215.73	219.08	223.10	218.25	220.20	223.85
	S.D.	.	7.05	8.64	7.41	6.73	7.86	7.85	6.72	4.46	8.45	6.33	2.96	4.02
	N	2	4	4	4	4	4	4	4	4	4	4	4	4
CF	Mean	213.75	201.65	210.38	206.78	211.48	211.98	208.38	209.20	209.65	212.13	214.83	213.15	213.68
	S.D.	.	.	3.97	9.51	10.80	10.66	5.74	11.49	15.56	6.54	11.12	9.68	6.88
	N	2	2	4	4	4	4	4	4	4	4	4	4	4
DF	Mean	207.65	211.13	211.15	206.53	205.85	196.23	195.20	192.48	201.08	202.30	196.73	201.45	201.48
	S.D.	.	4.91	6.51	6.79	10.21	5.13	9.61	9.28	6.98	12.80	14.06	11.69	14.86
	N	2	4	4	4	4	4	4	4	4	4	4	4	4
EF	Mean	.	203.95	213.40	196.15	189.75	184.30	178.90	169.50	161.50	153.05	145.30	140.05	142.00
	S.D.	.	.	.	.	.	.	.	.	.	.	.	.	.
	N	0	2	2	2	2	2	2	2	2	2	2	2	1
FF	Mean	209.45	.	218.05	211.95	215.65	215.65	215.95	210.20	215.20	218.55	218.15	216.85	220.45
	S.D.	.	.	.	.	.	.	.	.	.	.	.	.	.
	N	2	0	2	2	2	2	2	2	2	2	2	2	2

(.) = Not applicable
[a] All animals in group dead prior to scheduled collection period.
Group V - 0 mg/kg/day Group A - 10 mg/kg/day Artelinic Acid Group B - 20 mg/kg/day Artelinic Acid
Group C - 40 mg/kg/day Artelinic Acid Group D - 80 mg/kg/day Artelinic Acid Group E - 320 mg/kg/day Artelinic Acid
Group F - 12.5 mg/kg/day Arteether

Table 22 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Summary Body Weights (grams): Females

Group Sex		Day numbers relative to Start Date			
		12	13	14	21
VF	Mean	248.05	250.75	248.25	241.85
	S.D.	.	.	.	.
	N	2	2	2	2
AF	Mean	230.93	233.50	233.13	226.53
	S.D.	5.63	8.25	10.51	11.32
	N	4	4	4	4
BF	Mean	225.78	224.68	225.60	219.30
	S.D.	6.27	5.21	5.11	6.01
	N	4	4	4	4
CF	Mean	218.08	221.38	222.38	215.85
	S.D.	11.93	7.57	11.11	8.28
	N	4	4	4	4
DF	Mean	202.98	205.33	209.78	207.95
	S.D.	14.20	10.13	10.51	11.84
	N	4	4	4	4
EF	Mean	a			
	S.D.				
	N				
FF	Mean	224.40	225.40	224.05	215.30
	S.D.	.	.	.	.
	N	2	2	2	2

(.) = Not applicable
[a] All animals in group dead prior to scheduled collection period.
Group V - 0 mg/kg/day Group A - 10 mg/kg/day Artelinic Acid Group B - 20 mg/kg/day Artelinic Acid
Group C - 40 mg/kg/day Artelinic Acid Group D - 80 mg/kg/day Artelinic Acid Group E - 320 mg/kg/day Artelinic Acid
Group F - 12.5 mg/kg/day Arteether

Table 23

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01 SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	WBC	RBC	HGB	HCT
UNITS:	thds/mm3	mil/mm3	g/dL	%
Group: VM : 0 mg/kg/day				
MEAN	13.43	8.04	15.8	48.1
SD	2.708	0.389	0.42	2.40
N	2	2	2	2
Group: AM : 10 mg/kg/day Artelinic Acid				
MEAN	10.07	7.57	14.7	44.7
SD	2.103	1.027	1.07	4.00
N	4	4	4	4
Group: BM : 20 mg/kg/day Artelinic Acid				
MEAN	14.31	7.67	15.9	49.4
SD	1.562	0.275	0.65	1.81
N	4	4	4	4
Group: CM : 40 mg/kg/day Artelinic Acid				
MEAN	8.95	7.88	15.1	47.5
SD	1.888	0.672	0.14	0.28
N	2	2	2	2
Group: DM : 80 mg/kg/day Artelinic Acid				
MEAN	9.46	6.88	14.8	45.2
SD	0.997	0.279	0.51	2.29
N	3	3	3	3
Group: EM : 320 mg/kg/day Artelinic Acid				
MEAN	9.01	7.37	14.6	45.3
SD	0.467	0.332	0.07	1.20
N	2	2	2	2
Group: FM : 12.5 mg/kg/day Arteether				
MEAN	12.62	7.64	14.5	45.2
SD	2.425	0.262	0.99	4.03
N	2	2	2	2

Table 23 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01 SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	MCV	MCH	MCHC	PLT	RETIC
UNITS:	fL	pg	g/dL	thds/mm3	10**5/mm3
Group: VM : 0 mg/kg/day					
MEAN	59.8	19.6	32.8	1110	2.7
SD	0.14	0.42	0.85	66.5	0.35
N	2	2	2	2	2
Group: AM : 10 mg/kg/day Artelinic Acid					
MEAN	59.3	19.5	32.9	1058	2.7
SD	3.11	1.68	1.13	129.1	0.34
N	4	4	4	4	4
Group: BM : 20 mg/kg/day Artelinic Acid					
MEAN	64.4	20.7	32.2	1194	4.8
SD	1.07	0.63	0.56	107.2	1.63
N	4	4	4	4	4
Group: CM : 40 mg/kg/day Artelinic Acid					
MEAN	60.6	19.3	31.8	1246	5.1
SD	4.74	1.48	0.07	7.8	0.14
N	2	2	2	2	2
Group: DM : 80 mg/kg/day Artelinic Acid					
MEAN	65.8	21.4	32.7	1181	5.2
SD	4.19	0.15	2.01	115.7	1.95
N	3	3	3	3	3
Group: EM : 320 mg/kg/day Artelinic Acid					
MEAN	61.4	19.8	32.2	1048	8.6**
SD	1.13	0.92	0.92	347.9	0.28
N	2	2	2	2	2
Group: FM : 12.5 mg/kg/day Arteether					
MEAN	59.1	19.1	32.2	1056	2.9
SD	3.18	0.64	0.71	143.5	0.49
N	2	2	2	2	2

**-Significant Difference from Control P < .01

07-OCT-1999

Table 23 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01 SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	NEUT ABS	LYMPH ABS	MONO ABS	EOS ABS	BASO ABS	LUC ABS
UNITS:	thds/mm3	thds/mm3	thds/mm3	thds/mm3	thds/mm3	thds/mm3
Group: VM : 0 mg/kg/day						
MEAN	0.91	12.08	0.14	0.06	0.10	0.16
SD	0.057	2.680	0.035	0.000	0.007	0.064
N	2	2	2	2	2	2
Group: AM : 10 mg/kg/day Artelinic Acid						
MEAN	1.21	8.44	0.17	0.07	0.05	0.13
SD	0.366	1.774	0.116	0.006	0.024	0.033
N	4	4	4	4	4	4
Group: BM : 20 mg/kg/day Artelinic Acid						
MEAN	1.57	12.21	0.18	0.07	0.10	0.19
SD	0.416	1.085	0.057	0.045	0.030	0.031
N	4	4	4	4	4	4
Group: CM : 40 mg/kg/day Artelinic Acid						
MEAN	0.98	7.66	0.13	0.03	0.04	0.12
SD	0.530	1.273	0.049	0.000	0.014	0.021
N	2	2	2	2	2	2
Group: DM : 80 mg/kg/day Artelinic Acid						
MEAN	0.84	8.36	0.09	0.06	0.03*	0.08
SD	0.172	0.876	0.023	0.006	0.010	0.023
N	3	3	3	3	3	3
Group: EM : 320 mg/kg/day Artelinic Acid						
MEAN	1.00	7.69	0.16	0.02	0.04	0.12
SD	0.467	0.085	0.078	0.007	0.007	0.007
N	2	2	2	2	2	2
Group: FM : 12.5 mg/kg/day Arteether						
MEAN	1.09	11.15	0.11	0.08	0.07	0.13
SD	0.000	2.369	0.000	0.014	0.007	0.028
N	2	2	2	2	2	2

*-Significant Difference from Control P < .05

04-OCT-1999

Table 23 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01 SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	WBC	RBC	HGB	HCT
UNITS:	thds/mm3	mil/mm3	g/dL	%
Group: VF : 0 mg/kg/day				
MEAN	6.96	7.70	14.2	43.8
SD	3.352	0.962	1.34	5.30
N	2	2	2	2
Group: AF : 10 mg/kg/day Artelinic Acid				
MEAN	8.49	6.87	13.0	39.3
SD	1.686	1.134	2.02	6.44
N	4	4	4	4
Group: BF : 20 mg/kg/day Artelinic Acid				
MEAN	10.44	7.85	15.0	46.1
SD	4.460	0.162	0.19	0.67
N	4	4	4	4
Group: CF : 40 mg/kg/day Artelinic Acid				
MEAN	7.81	7.30	14.1	42.3
SD	2.723	0.668	1.25	3.90
N	4	4	4	4
Group: DF : 80 mg/kg/day Artelinic Acid				
MEAN	10.03	6.46	13.1	40.2
SD	2.904	0.945	2.17	6.61
N	4	4	4	4
Group: EF : 320 mg/kg/day Artelinic Acid				
MEAN	NA	NA	NA	NA
SD	NA	NA	NA	NA
N	0	0	0	0
Group: FF : 12.5 mg/kg/day Arteether				
MEAN	9.18	6.98	13.3	40.3
SD	0.891	1.259	3.04	8.70
N	2	2	2	2

NA-Not Applicable

04-OCT-1999

Table 23 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01 SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	MCV	MCH	MCHC	PLT	RETIC
UNITS:	fL	pg	g/dL	thds/mm3	10**5/mm3
Group: VF : 0 mg/kg/day					
MEAN	56.8	18.4	32.4	1171	1.9
SD	0.14	0.57	0.92	224.9	0.14
N	2	2	2	2	2
Group: AF : 10 mg/kg/day Artelinic Acid					
MEAN	57.3	19.0	33.2	1040	3.0
SD	0.36	0.74	1.08	265.1	0.71
N	4	4	4	4	4
Group: BF : 20 mg/kg/day Artelinic Acid					
MEAN	58.8	19.2	32.6	1105	4.4
SD	1.18	0.44	0.17	213.9	0.78
N	4	4	4	4	4
Group: CF : 40 mg/kg/day Artelinic Acid					
MEAN	58.0	19.3	33.2	1202	4.3
SD	0.73	0.05	0.46	226.2	0.87
N	4	4	4	4	4
Group: DF : 80 mg/kg/day Artelinic Acid					
MEAN	62.1*	20.4	32.8	1081	6.0**
SD	3.35	1.50	0.97	52.2	2.11
N	4	4	4	4	4
Group: EF : 320 mg/kg/day Artelinic Acid					
MEAN	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA
N	0	0	0	0	0
Group: FF : 12.5 mg/kg/day Arteether					
MEAN	57.5	18.9	32.9	1036	2.2
SD	2.12	0.99	0.64	19.8	0.28
N	2	2	2	2	2

*-Significant Difference from Control P < .05 NA-Not Applicable
**-Significant Difference from Control P < .01

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Table 23 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

SUMMARY HEMATOLOGY REPORT
PERIOD: Day 21

STUDY ID: 9610.03.01 SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	NEUT ABS	LYMPH ABS	MONO ABS	EOS ABS	BASO ABS	LUC ABS
UNITS:	thds/mm3	thds/mm3	thds/mm3	thds/mm3	thds/mm3	thds/mm3
Group: VF : 0 mg/kg/day						
MEAN	0.74	5.87	0.14	0.08	0.03	0.12
SD	0.516	2.496	0.113	0.092	0.028	0.106
N	2	2	2	2	2	2
Group: AF : 10 mg/kg/day Artelinic Acid						
MEAN	1.20	6.95	0.09	0.12	0.04	0.10
SD	0.834	1.448	0.053	0.095	0.026	0.029
N	4	4	4	4	4	4
Group: BF : 20 mg/kg/day Artelinic Acid						
MEAN	0.96	9.02	0.15	0.07	0.06	0.19
SD	0.691	3.977	0.102	0.021	0.031	0.165
N	4	4	4	4	4	4
Group: CF : 40 mg/kg/day Artelinic Acid						
MEAN	1.56	5.76	0.20	0.06	0.03	0.20
SD	1.543	1.709	0.139	0.022	0.013	0.133
N	4	4	4	4	4	4
Group: DF : 80 mg/kg/day Artelinic Acid						
MEAN	0.98	8.59	0.18	0.06	0.05	0.18
SD	0.820	2.493	0.031	0.024	0.026	0.053
N	4	4	4	4	4	4
Group: EF : 320 mg/kg/day Artelinic Acid						
MEAN	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0
Group: FF : 12.5 mg/kg/day Arteether						
MEAN	1.22	7.70	0.08	0.08	0.05	0.07
SD	0.615	0.269	0.021	0.000	0.007	0.028
N	2	2	2	2	2	2

NA-Not Applicable

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Table 24

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

SUMMARY CLINICAL CHEMISTRY REPORT
PERIOD: DAY 21

STUDY ID: 9610.03.01 SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	NA	K	CL	ALP	ALT	AST
UNITS:	mmol/L	mmol/L	mmol/L	U/L	U/L	U/L
Group: VM : 0 mg/kg/day						
MEAN	147	6.5	97	227	40	76
SD	1.4	0.21	2.8	24.7	12.0	7.8
N	2	2	2	2	2	2
Group: AM : 10 mg/kg/day Artelinic Acid						
MEAN	147	6.2	99	156	35	86
SD	0.5	0.62	2.2	35.0	7.0	9.3
N	4	4	4	4	4	4
Group: BM : 20 mg/kg/day Artelinic Acid						
MEAN	146	6.7	100	159	38	107
SD	0.8	0.47	0.8	36.8	2.9	18.1
N	4	4	4	4	4	4
Group: CM : 40 mg/kg/day Artelinic Acid						
MEAN	147	6.5	99	148	38	95
SD	1.3	0.29	2.4	31.3	8.4	10.8
N	4	4	4	4	4	4
Group: DM : 80 mg/kg/day Artelinic Acid						
MEAN	145	6.7	100	162	48	99
SD	1.4	0.60	1.8	65.1	7.7	18.0
N	4	4	4	4	4	4
Group: EM : 320 mg/kg/day Artelinic Acid						
MEAN	146	6.7	99	111	38	111
SD	0.0	0.57	0.7	72.1	7.1	21.9
N	2	2	2	2	2	2
Group: FM : 12.5 mg/kg/day Arteether						
MEAN	146	6.4	100	179	38	87
SD	0.0	0.49	0.7	43.8	7.8	6.4
N	2	2	2	2	2	2

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Table 24 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

SUMMARY CLINICAL CHEMISTRY REPORT
PERIOD: DAY 21

STUDY ID: 9610.03.01 SEX: MALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	BUN	CREA	BUN/CRE	GLUC	TP	ALB
UNITS:	mg/dL	mg/dL	ratio	mg/dL	g/dL	g/dL
Group: VM : 0 mg/kg/day						
MEAN	23.3	0.6	42.3	89	7.0	4.9
SD	2.76	0.07	0.42	7.8	0.14	0.14
N	2	2	2	2	2	2
Group: AM : 10 mg/kg/day Artelinic Acid						
MEAN	16.7	0.6	31.2	87	6.6	4.8
SD	3.62	0.10	8.66	11.0	0.24	0.17
N	4	4	4	4	4	4
Group: BM : 20 mg/kg/day Artelinic Acid						
MEAN	18.3	0.6	31.8	85	6.6	4.8
SD	4.50	0.05	6.70	9.4	0.22	0.10
N	4	4	4	4	4	4
Group: CM : 40 mg/kg/day Artelinic Acid						
MEAN	16.9	0.6	29.2	71	6.6	4.8
SD	3.59	0.05	4.76	30.2	0.17	0.13
N	4	4	4	4	4	4
Group: DM : 80 mg/kg/day Artelinic Acid						
MEAN	18.4	0.6	33.7	92	6.5	4.7
SD	4.69	0.06	9.65	15.7	0.30	0.22
N	4	4	4	4	4	4
Group: EM : 320 mg/kg/day Artelinic Acid						
MEAN	25.1	0.6	45.5	122	6.6	4.8
SD	4.81	0.07	2.90	7.1	0.64	0.35
N	2	2	2	2	2	2
Group: FM : 12.5 mg/kg/day Arteether						
MEAN	21.6	0.5	43.6	108	6.6	4.8
SD	4.45	0.14	3.39	4.2	0.28	0.21
N	2	2	2	2	2	2

Table 24 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

SUMMARY CLINICAL CHEMISTRY REPORT
PERIOD: DAY 21

STUDY ID: 9610.03.01 SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	NA	K	CL	ALP	ALT	AST
UNITS:	mmol/L	mmol/L	mmol/L	U/L	U/L	U/L
Group: VF : 0 mg/kg/day						
MEAN	145	5.9	98	108	30	83
SD	0.7	0.35	0.7	24.7	0.0	6.4
N	2	2	2	2	2	2
Group: AF : 10 mg/kg/day Artelinic Acid						
MEAN	145	5.8	99	78	30	80
SD	1.7	0.53	2.2	26.3	6.9	6.6
N	4	4	4	4	4	4
Group: BF : 20 mg/kg/day Artelinic Acid						
MEAN	145	6.0	99	77	32	104
SD	0.6	0.33	1.9	6.0	16.7	25.1
N	4	4	4	4	4	4
Group: CF : 40 mg/kg/day Artelinic Acid						
MEAN	145	6.0	99	83	33	91
SD	1.0	0.50	2.6	13.3	11.1	4.6
N	4	4	4	4	4	4
Group: DF : 80 mg/kg/day Artelinic Acid						
MEAN	145	6.3	102	110	30	108
SD	1.0	0.54	2.6	13.4	8.6	11.5
N	4	4	4	4	4	4
Group: EF : 320 mg/kg/day Artelinic Acid						
MEAN	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0
Group: FF : 12.5 mg/kg/day Arteether						
MEAN	145	5.9	100	94	28	97
SD	0.0	0.28	1.4	7.8	4.2	7.8
N	2	2	2	2	2	2

NA-Not Applicable

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Table 24 (Continued)

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

SUMMARY CLINICAL CHEMISTRY REPORT
PERIOD: DAY 21

STUDY ID: 9610.03.01 SEX: FEMALE

ANALYSIS OF VARIANCE FOLLOWED BY DUNNETT'S PROCEDURE

TEST(s):	BUN	CREA	BUN/CRE	GLUC	TP	ALB
UNITS:	mg/dL	mg/dL	ratio	mg/dL	g/dL	g/dL
Group: VF : 0 mg/kg/day						
MEAN	13.4	0.7	19.1	89	7.2	5.4
SD	2.97	0.00	4.24	6.4	0.35	0.28
N	2	2	2	2	2	2
Group: AF : 10 mg/kg/day Artelinic Acid						
MEAN	16.5	0.6	29.0	92	7.3	5.4
SD	3.12	0.05	7.37	14.0	0.30	0.29
N	4	4	4	4	4	4
Group: BF : 20 mg/kg/day Artelinic Acid						
MEAN	19.6	0.6	32.9	90	7.6	5.7
SD	2.99	0.12	1.59	2.6	0.36	0.28
N	4	4	4	4	4	4
Group: CF : 40 mg/kg/day Artelinic Acid						
MEAN	22.0	0.6	35.1	85	7.5	5.5
SD	4.55	0.13	2.83	11.1	0.48	0.36
N	4	4	4	4	4	4
Group: DF : 80 mg/kg/day Artelinic Acid						
MEAN	17.7	0.6	31.1	84	6.7	5.1
SD	6.87	0.05	12.83	20.5	0.22	0.19
N	4	4	4	4	4	4
Group: EF : 320 mg/kg/day Artelinic Acid						
MEAN	NA	NA	NA	NA	NA	NA
SD	NA	NA	NA	NA	NA	NA
N	0	0	0	0	0	0
Group: FF : 12.5 mg/kg/day Arteether						
MEAN	19.0	0.7	29.2	82	7.0	5.1
SD	2.40	0.07	0.57	9.2	0.14	0.21
N	2	2	2	2	2	2

NA-Not Applicable

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Table 25

Effect of Artelinic Acid on Rats after Oral Administration for 14 Days

Histopathological Findings

Vehicle Control	
3M-9	Normal
3M-10	Normal
3F-31	Normal
3F-32	Normal
Arteether	
7M-21	Neuron degeneration +1,
7M-22	Neuron degeneration +2, gliosis +1
7F-43	Neuron degeneration +1, several suspicious cells
7F-44	Neuron degeneration +1
10 mg/kg Artelinic Acid	
6M-17	Normal
6M-18	Normal
6M-19	Normal
6M-20	Normal
6F-39	Trapezoid nucleus not identified
6F-40	Normal
6F-41	Normal
6F-42	Normal
20 mg/kg Artelinic Acid	
1M-1	Normal
1M-2	Normal
1M-3	Normal
1M-4	Normal
1F-23	Normal
1F-24	Normal
1F-25	Normal
1F-26	Normal
40 mg/kg Artelinic Acid	
4M-11	Incomplete perfusion, no lesions seen
4M-12	Normal
4M-13	Normal
4M-14	Normal
4F-33	Neuron degeneration, +1
4F-34	Normal
4F-35	Neuron degeneration +1
4F-36	Neuron degeneration +1
80 mg/kg Artelinic Acid	
2M-5	Neuron degeneration, +2
2M-6	Neuron degeneration, +2
2M-7	Neuron degeneration, +2
2M-8	Neuron degeneration, +2
2F-27	Neuron degeneration, +1
2F-28	Neuron degeneration, +2
2F-29	Neuron degeneration, +1
2F-30	Neuron degeneration, +1
320 mg/kg Artelinic Acid	
5M-15	Neuron degeneration, +4, multiple nuclei in hind brain
5M-16	Neuron degeneration, +4, multiple nuclei in hind brain
5F-37	Died on study, not perfused, possible neuron degeneration
5F-38	Died on study, not perfused

Table 26

Effect of Artelinic Acid on Dogs after Oral Administration for 14 Days

Dose Formulation/Homogeneity Analysis: Artelinic Acid

Dose Level (mg/kg/day)	Analysis Date	Concentration (mg/mL)									
		Theoretical	Actual						Sample Average	Percent (%) RSD	Percent (%) Theoretical[a]
			Sample No.								
			1	2	3	4	5	6			
20	10/04/99[b]	2.00	1.93	1.94	1.90	NA	NA	NA	1.92	1.0	96.1
40	10/05/99[b]	4.00	3.68	3.64	3.42	NA	NA	NA	3.58	3.9	89.6
80	10/04/99[b]	8.00	6.08	7.55	7.83	NA	NA	NA	7.16	13	89.4
320	10/04/99[b]	32.0	32.0	31.9	31.2	NA	NA	NA	31.7	1.4	99.0
20	10/11/99[b]	2.00	1.57	1.52	1.60	NA	NA	NA	1.56	2.6	78.0[d]
20	10/13/99[c]	2.00	1.72	1.73	1.60	1.58	1.46	1.57	1.61	6.3	80.6
40	10/11/99[b]	4.00	3.65	3.76	3.63	NA	NA	NA	3.68	2.0	92.0
80	10/11/99[b]	8.00	7.54	7.61	7.09	NA	NA	NA	7.41	3.8	92.7
320	10/11/99[b]	32.0	24.3	27.4	26.2	NA	NA	NA	26.0	6.0	81.1[d]
320	10/13/99[c]	32.0	27.0	32.7	31.5	30.8	32.7	31.0	30.9	6.8	96.7

NA = not applicable

RSD = relative standard deviation

All calculated values reported are based upon nontruncated numbers and may not be reproducible based upon the rounded values that appear in the table.

[a] Percent Theoretical = $\frac{\text{Actual Concentration}}{\text{Theoretical Concentration}} \times 100$

[b] Homogeneity samples taken from the top (Sample 1), middle (Sample 2), and bottom (Sample 3) of the formulation.

[c] Samples were taken from six separate bottles that contained the same formulation.

Table 27

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Males

Group Sex	Dose Level	Animal Number	Body Weights (kg) on Study Day								
			-4	1	2	3	4	5	6	7	8
VM	0 mg/kg/day	2598	5.9	5.9	5.7	5.7	5.8	5.7	5.8	5.7	5.8
		2594	7.6	7.6	7.5	7.7	7.5	7.9	7.7	7.6	7.6
Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21	
VM	0 mg/kg/day	2598	6.1	6.0	5.9	6.1	6.1	6.1	NA	5.9	
		2594	7.8	7.6	7.5	7.9	7.7	8.0	7.8	[a]	
Group Sex	Dose Level	Animal Number	-4	1	2	3	4	5	6	7	8
AM	20 mg/kg/day Artelinic Acid	2599	7.0	7.1	7.0	6.8	6.9	6.9	6.9	6.9	6.9
		2602	6.7	6.5	6.5	6.4	6.3	6.4	6.2	6.3	6.3
Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21	
AM	20 mg/kg/day Artelinic Acid	2599	7.0	6.9	6.9	6.9	6.9	6.9	6.8	[a]	
		2602	6.6	6.5	6.4	6.3	6.3	6.6	NA	6.3	

NA = Not applicable

[a] Animal dead prior to data collection period.

Table 27 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Males

Group Sex	Dose Level	Animal Number	Body Weights (kg) on Study Day								
			-4	1	2	3	4	5	6	7	8
BM	40 mg/kg/day Artelinic Acid	2600	6.7	6.7	6.6	6.5	6.5	6.6	6.5	6.4	6.5
		2595	7.7	7.7	7.9	7.9	7.8	7.8	7.9	7.9	7.7

Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21
BM	40 mg/kg/day Artelinic Acid	2600	6.5	6.4	6.4	6.4	6.4	6.5	NA	6.3
		2695	7.8	7.8	7.8	7.9	7.8	7.9	7.7	a

Group Sex	Dose Level	Animal Number	-4	1	2	3	4	5	6	7	8
CM	80 mg/kg/day Artelinic Acid	2604	6.1	6.0	6.2	5.9	6.2	5.9	5.8	5.7	5.9
		2596	6.8	6.6	6.5	6.5	6.6	6.5	6.4	6.6	6.5

Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21
CM	80 mg/kg/day Artelinic Acid	2604	6.1	5.8	5.7	5.8	5.6	5.9	NA	5.6
		2596	6.5	6.4	6.3	6.5	6.3	6.4	6.4	a

NA = Not applicable

[a] Animal dead prior to data collection period.

Table 27 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Males

Group Sex	Dose Level	Animal Number	Body Weights (kg) on Study Day -4	1	2	3	4	5	6	7	8
DM	320 mg/kg/day Artelinic Acid	2605	6.3	6.1	5.9	5.8	5.9	5.6	5.7	5.6	5.5
		2601	7.2	7.0	7.0	6.7	6.8	6.4	6.5	6.3	6.2

Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21
DM	320 mg/kg/day Artelinic Acid	2605	5.4	5.7	5.6	5.5	5.4	5.5	NA	5.6
		2601	6.3	6.3	6.2	6.2	6.1	6.3	5.9	a

Group Sex	Dose Level	Animal Number	-4	1	2	3	4	5	6	7	8
EM	20 mg/kg/day Arteether	2597	7.8	7.8	7.6	7.6	7.5	7.5	7.5	7.4	7.4
		2603	6.6	6.5	6.4	6.5	6.6	6.4	6.6	6.7	6.8

Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21
EM	20 mg/kg/day Arteether	2597	7.4	7.2	7.1	7.1	7.2	7.2	7.0	a
		2603	6.9	6.8	6.7	6.9	6.9	7.1	NA	6.8

NA = Not applicable

[a] Animal dead prior to data collection period.

Table 27 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Females

Group Sex	Dose Level	Animal Number	Body Weight (kg) on Study Day -5	1	2	3	4	5	6	7	8
VF	0 mg/kg/day	2612	5.3	5.1	5.1	5.2	5.1	5.1	5.0	5.1	5.1
		2611	6.4	6.2	6.3	6.2	6.1	6.2	6.1	6.1	6.4

Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21
VF	0 mg/kg/day	2612	5.1	5.0	5.2	5.1	5.2	5.2	NA	5.1
		2611	6.1	6.2	6.2	6.1	6.1	6.3	6.0	a

Group Sex	Dose Level	Animal Number	-5	1	2	3	4	5	6	7	8
AF	20 mg/kg/day Artelinic Acid	2618	5.6	5.3	5.4	5.3	5.3	5.2	5.5	5.5	5.4
		2614	5.8	5.6	5.7	5.6	5.5	5.7	5.6	5.6	5.7

Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21
AF	20 mg/kg/day Artelinic Acid	2618	5.3	5.2	5.4	5.2	5.2	5.3	NA	5.7
		2614	5.8	5.6	5.7	5.6	5.7	5.6	5.6	a

NA = Not applicable

[a] Animal dead prior to data collection period.

Table 27 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Females

Group Sex	Dose Level	Animal Number	Body Weights (kg) on Study Day								
			-5	1	2	3	4	5	6	7	8
BF	40 mg/kg day Artelinic Acid	2616	6.2	6.2	6.2	6.4	6.1	6.1	6.1	6.2	6.2
		2606	5.8	5.6	5.5	5.5	5.3	5.6	5.5	5.6	5.7

Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21
BF	40 mg/kg/day Artelinic Acid	2616	6.1	6.1	6.2	6.1	6.3	6.3	NA	6.4
		2606	5.4	5.5	5.5	5.3	5.5	5.5	5.2	a

Group Sex	Dose Level	Animal Number	-5	1	2	3	4	5	6	7	8
CF	80 mg/kg/day Artelinic Acid	2609	5.4	5.3	5.3	5.3	5.2	5.2	5.2	5.2	5.2
		2587	7.3	7.3	7.2	7.3	7.2	7.2	7.2	7.3	7.2

Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21
CF	80 mg/kg/day Artelinic Acid	2609	5.2	5.1	5.2	5.1	5.3	5.2	NA	5.4
		2587	7.1	7.1	7.3	7.2	7.2	7.0	7.0	a

NA = Not applicable

[a] Animal dead prior to data collection period.

Table 27 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Individual Body Weights: Females

Group Sex	Dose Level	Animal Number	Body Weights (kg) on Study Day -5	1	2	3	4	5	6	7	8
DF	320 mg/kg/day Artelinic Acid	2608	5.8	5.9	5.7	5.8	5.6	5.5	5.4	5.3	5.2
		2607	5.8	6.0	5.7	5.6	5.6	a			

Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21
DF	320 mg/kg/day Artelinic Acid	2608	5.1	5.0	5.0	4.8	4.7	4.6	4.5	5.1
		2607								

Group Sex	Dose Level	Animal Number	-5	1	2	3	4	5	6	7	8
EF	20 mg/kg/day Arteether	2615	5.6	5.4	5.6	5.6	5.5	5.4	5.5	5.3	5.4
		2610	6.8	6.6	6.7	6.7	6.5	6.5	6.5	6.5	6.5

Group Sex	Dose Level	Animal Number	9	10	11	12	13	14	15	21
EF	20 mg/kg/day Arteether	2615	5.2	5.0	4.9	4.8	4.9	4.9	4.6	a
		2610	6.3	6.1	5.8	5.6	5.4	5.3	5.2	a

NA = Not applicable

[a] Animal dead prior to data collection period.

Table 28

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Plasma Concentrations of Artelinic Acid

Day 1	Plasma Concentration (ng/mL)											
Dose:	0 mg/kg/day				20 mg/kg/day				40 mg/kg/day			
	Males		Females		Males		Females		Males		Females	
Time Point	VM2594	VM2598	VF2611	VF2612	AM2599	AM2602	AF2614	AF2618	BM2595	BM2600	BF2606	BF2616
0	NA	NA	NA	NA	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
0.25	BQL	BQL	BQL	175	9500	7620	9010	2320	5510	9260	13100	19000
0.5	NA	NA	NA	NA	11300	10800	13900	7060	13400	19600	26700	21900
1	NA	NA	NA	NA	10900	5430	9530	9440	22100	16800	22500	18300
2	NA	NA	NA	NA	1630	481	866	845	5930	7170	2090	3610
4	NA	NA	NA	NA	14.3	24.1	28.3	82.4	196	557	46.4	171
8	NA	NA	NA	NA	BQL	BQL	BQL	BQL	BQL	BQL	BQL	6.95
Day 7	Plasma Concentration (ng/mL)											
Dose:	0 mg/kg/day				20 mg/kg/day				40 mg/kg/day			
	Males		Females		Males		Females		Males		Females	
Time Point	VM2594	VM2598	VF2611	VF2612	AM2599	AM2602	AF2614	AF2618	BM2595	BM2600	BF2606	BF2616
0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL

NA = Not applicable; analytical results were obtained only for samples collected at 0.25 hours after dosing

BQL = Below quantification limit (below assay sensitivity; lower limit of quantification = 4 ng/mL)

D = Animal dead prior to scheduled sample collection.

Table 28 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Plasma Concentrations of Artelinic Acid

Day 14	Plasma Concentration (ng/mL)											
Dose:	0 mg/kg/day				20 mg/kg/day				40 mg/kg/day			
	Males		Females		Males		Females		Males		Females	
Time Point	VM2594	VM2598	VF2611	VF2612	AM2599	AM2602	AF2614	AF2618	BM2595	BM2600	BF2606	BF2616
0	NA	NA	NA	NA	BQL	BQL	4.62	BQL	BQL	BQL	BQL	BQL
0.25	BQL	BQL	5.97	BQL	13200	4930	5130	11100	4110	10200	17000	13100
0.5	NA	NA	NA	NA	15300	6680	8140	13000	10700	18800	26400	15800
1	NA	NA	NA	NA	8220	4900	5850	6320	16700	23100	13500	15200
2	NA	NA	NA	NA	1230	921	1440	262	3440	3260	1600	2620
4	NA	NA	NA	NA	52.3	128	323	13.6	112	171	165	120
8	NA	NA	NA	NA	BQL	BQL	BQL	BQL	4.93	8.56	13.6	4.02

NA = Not applicable; analytical results were obtained only for samples collected at 0.25 hours after dosing

BQL = Below quantification limit (below assay sensitivity; lower limit of quantification = 4 ng/mL)

D = Animal dead prior to scheduled sample collection.

Table 28 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Plasma Concentrations of Artelinic Acid

Day 1	Plasma Concentration (ng/mL)							
Dose:	80 mg/kg/day				320 mg/kg/day			
	Males		Females		Males		Females	
Time Point	CM2596	CM2604	CF2587	CF2609	DM2601	DM2605	DF2608	DF2607
0	BQL	BQL	BQL	BQL	BQL	BQL	BQL	BQL
0.25	19900	18000	15700	5950	102000	31700	39400	29700
0.5	28900	35100	32500	17000	160000	89100	53100	60800
1	23700	30500	35300	32500	111000	119000	48000	90300
2	6650	4730	16900	11000	40500	69400	19100	35800
4	259	548	2050	1360	2830	8150	1950	2210
8	5.86	84.9	11.7	13.1	1560	392	571	942
Day 7	Plasma Concentration (ng/mL)							
Dose:	80 mg/kg/day				320 mg/kg/day			
	Males		Females		Males		Females	
Time Point	CM2596	CM2604	CF2587	CF2609	DM2601	DM2605	DF2608	DF2607
0	BQL	BQL	BQL	BQL	7.88	5.64	7.21	D

NA = Not applicable; analytical results were obtained only for samples collected at 0.25 hours after dosing
BQL = Below quantification limit (below assay sensitivity; lower limit of quantification = 4 ng/mL)
D = Animal dead prior to scheduled sample collection.

Table 28 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Plasma Concentrations of Artelinic Acid

Day 14	Plasma Concentration (ng/mL)							
Dose:	80 mg/kg/day				320 mg/kg/day			
	Males		Females		Males		Females	
Time Point	CM2596	CM2604	CF2587	CF2609	DM2601	DM2605	DF2608	DF2607
0	BQL	BQL	BQL	BQL	BQL	BQL	172	D
0.25	16200	31700	9500	7820	48700	39000	33900	D
0.5	16300	42700	18200	14200	90800	74200	47800	D
1	11100	26600	23900	28800	67500	68000	26300	D
2	1580	3150	5130	5770	12600	16100	14400	D
4	236	222	544	474	4710	1140	4590	D
8	63.5	26	140	76.3	444	66.2	523	D

NA = Not applicable; analytical results were obtained only for samples collected at 0.25 hours after dosing

BQL = Below quantification limit (below assay sensitivity; lower limit of quantification = 4 ng/mL)

D = Animal dead prior to scheduled sample collection.

Table 29

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Pharmacokinetic Parameters Calculated from Plasma Concentrations of Artelinic Acid

Noncompartmental analysis (w=1) using WinNonlin 1.1

	Day 1						Day 14					
Dog ID	AM2599	AM2602	AF2614	AF2618	Mean	SD	AM2599	AM2602	AF2614	AF2618	Mean	SD
Oral Dose (mg/kg)	20						20					
$t_{1/2z1}$ (hr)	0.31	0.39	0.36	0.46	0.38	0.06	0.41	0.59	0.74	0.35	0.52	0.18
$t_{1/2z2}$ (hr)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
$AUC_{0\text{-last}}$ (ng*h/mL)	17247	10773	15940	11657	13904	3171	17100	8922	11206	12797	12506	3451
$AUC_{0\text{-last,N}}$ (ng*h/mL)	862	539	797	583	**695**	**159**	855	446	560	640	**625**	**173**
$AUC_{0\text{-inf}}$ (ng*h/mL)	17253	10787	15955	11712	13927	3159	17131	9030	11552	12804	12629	3387
$AUC_{0\text{-inf,N}}$ (ng*h/mL)	863	539	798	586	**696**	**158**	857	452	578	640	**631**	**169**
C_{max} (ng/mL)	11300	10800	13900	9440	11360	1867	15300	6680	8140	13000	10780	4047
T_{max} (h)	0.50	0.50	0.50	1.00	0.63	0.25	0.50	0.50	0.50	0.50	0.50	0.00
MRT (h)	0.92	0.75	0.82	0.98	0.87	0.10	0.80	0.95	1.06	0.68	0.87	0.17

Dog ID	BM2595	BM2600	BF2606	BF2616	Mean	SD	BM2595	BM2600	BF2606	BF2616	Mean	SD
Oral Dose (mg/kg)	40						40					
$t_{1/2z1}$ (hr)	0.44	0.60	0.34	NA	0.46	0.13	NA	NA	NA	NA	NA	NA
$t_{1/2z2}$ (hr)	NA	NA	NA	0.87	0.87	NA	0.89	0.93	1.11	0.82	0.94	0.12
$AUC_{0\text{-last}}$ (ng*h/mL)	32069	33577	33344	32629	32905	688	23071	32345	27197	24898	26878	4017
$AUC_{0\text{-last,N}}$ (ng*h/mL)	802	839	834	816	**823**	**17**	577	809	680	622	**672**	**100**
$AUC_{0\text{-inf}}$ (ng*h/mL)	32191	34059	33367	32638	33064	822	23077	32357	27219	24903	26889	4020
$AUC_{0\text{-inf,N}}$ (ng*h/mL)	805	851	834	816	**827**	**21**	577	809	680	623	**672**	**101**
C_{max} (ng/mL)	22100	19600	26700	21900	22575	2975	16700	23100	26400	15800	20500	5102
T_{max} (h)	1.00	0.50	0.50	0.50	0.63	0.25	1.00	1.00	0.50	0.50	0.75	0.29
MRT (h)	1.19	1.21	0.87	0.98	1.06	0.17	1.15	1.03	0.85	0.99	1.01	0.12

$AUC_{0\text{-last,N}}$ (ng*h/mL): AUC normalized by dose to 1 mg/kg

$AUC_{0\text{-inf,N}}$ (ng*h/mL): AUC normalized by dose to 1 mg/kg

NS: no sample

NA: not available

t1/2z1 (hr): half-life for the first phase

t1/2z2 (hr): half-life for the second phase

Table 29 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Pharmacokinetic Parameters Calculated from Plasma Concentrations of Artelinic Acid

Noncompartmental analysis (w=1) using WinNonlin 1.1

Dog ID	CM2596	CM2604	CF2587	CF2609	Mean	SD	CM2596	CM2604	CF2587	CF2609	Mean	SD
Oral Dose (mg/kg)	80						80					
$t_{1/2z1}$ (hr)	NA	NA	0.57	0.61	0.59	0.03	NA	NA	NA	NA	NA	NA
$t_{1/2z2}$ (hr)	0.73	1.49	NA	NA	1.11	0.54	2.11	1.29	2.04	1.52	1.74	0.40
$AUC_{0\text{-last}}$ (ng*h/mL)	44351	49446	74111	52844	55188	13089	21693	49331	36732	39110	36717	11410
$AUC_{0\text{-last,N}}$ (ng*h/mL)	554	618	926	661	**690**	**164**	271	617	459	489	**459**	**143**
$AUC_{0\text{-inf}}$ (ng*h/mL)	44357	49628	74120	52855	55240	13065	21886	49379	37145	39277	36922	11356
$AUC_{0\text{-inf,N}}$ (ng*h/mL)	554	620	927	661	**691**	**163**	274	617	464	491	**462**	**142**
C_{max} (ng/mL)	28900	35100	35300	32500	32950	2986	16300	42700	23900	28800	27925	11112
T_{max} (h)	0.50	0.50	1.00	1.00	0.75	0.29	0.50	0.50	1.00	1.00	0.75	0.29
MRT (h)	1.07	1.07	1.47	1.47	1.27	0.23	0.97	0.86	1.25	1.25	1.08	0.20
Dog ID	DM2601	DM2605	DF2607	DF2608	Mean	SD	DM2601	DM2605	DF2607	DF2608	Mean	SD
Oral Dose (mg/kg)	320						320					
$t_{1/2z1}$ (hr)	NA	0.82	NA	NA	NA	NA	NA	NA	NS	NA	NA	NA
$t_{1/2z2}$ (hr)	4.66	NA	3.25	2.26	3.39	1.21	1.17	0.97	NS	1.28	1.14	0.16
$AUC_{0\text{-last}}$ (ng*h/mL)	241110	259922	160164	101405	190650	73573	130768	116277	NS	82563	109869	24733
$AUC_{0\text{-last,N}}$ (ng*h/mL)	753	812	501	317	**596**	**230**	409	363	NS	258	**343**	**77**
$AUC_{0\text{-inf}}$ (ng*h/mL)	251587	260384	164583	103264	194955	74873	131520	116370	NS	83526	110472	24535
$AUC_{0\text{-inf,N}}$ (ng*h/mL)	786	814	514	323	**609**	**234**	411	364	NS	261	**345**	**77**
C_{max} (ng/mL)	160000	119000	90300	53100	105600	45201	90800	74200	NS	47800	70933	21685
T_{max} (h)	0.50	1.00	1.00	0.50	0.75	0.29	0.50	0.50	NS	0.50	0.50	0.00
MRT (h)	1.24	1.62	1.44	1.36	1.42	0.16	1.32	1.12	NS	1.66	1.37	0.27

$AUC_{0\text{-last,N}}$ (ng*h/mL): AUC normalized by dose to 1 mg/kg

$AUC_{0\text{-inf,N}}$ (ng*h/mL): AUC normalized by dose to 1 mg/kg

NS: no sample

NA: not available

t1/2z1 (hr): half-life for the first phase

t1/2z2 (hr): half-life for the second phase

Table 30

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Pharmacokinetic Parameters Calculated from Plasma Concentrations of Arteether

	Day 1						Day 14					
Dog ID	EM2597	EM2603	EF2610	EF2615	Mean	SD	EM2597	EM2603	EF2610	EF2615	Mean	SD
IM Dose (mg/kg)	20						20					
$t_{1/2z1}$ (hr)	7.39	7.88	8.18	7.13	7.65	0.47	6.21	5.02	8.07	5.29	6.15	1.38
$AUC_{0\text{-}last}$ (ng*h/mL)	346	272	337	268	306	41	1425	1437	1270	1102	1309	157
$AUC_{0\text{-}inf}$ (ng*h/mL)	787	662	831	605	721	106	2333	2192	2551	1683	2190	369
C_{max} (ng/mL)	80.8	83.4	79.7	75.1	79.8	3.5	300.0	262.5	247.5	271.0	270.3	22.1
T_{max} (h)	2.0	1.0	2.0	1.0	1.5	0.6	0.5	2.0	1.0	0.5	1.00	0.71
$MRT_{0\text{-}last}$ (hr)	3.43	3.52	3.54	3.51	3.50	0.05	3.33	3.22	3.38	3.17	3.28	0.10

Noncompartmental analysis (w=1) using WinNonlin 1.1

Table 31

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Microscopic Lesions: Brain Tissues

Group	Sex	Treatment	Animal Number	Diagnosis and/or Comment	Severity
Artelinic Acid PO					
V	M	0 mg/kg/day	2595	Within Normal Limits	
V	M	0 mg/kg/day	2600	Within Normal Limits	
V	F	0 mg/kg/day	2606	Within Normal Limits	
V	F	0 mg/kg/day	2616	Within Normal Limits	
A	M	20 mg/kg/day	2594	Within Normal Limits	
A	M	20 mg/kg/day	2598	Within Normal Limits	
A	F	20 mg/kg/day	2611	Within Normal Limits	
A	F	20 mg/kg/day	2612	Within Normal Limits	
B	M	40 mg/kg/day	2601	Within Normal Limits	
B	M	40 mg/kg/day	2605	Within Normal Limits	
B	F	40 mg/kg/day	2607	Within Normal Limits	
B	F	40 mg/kg/day	2608	Within Normal Limits	
C	M	80 mg/kg/day	2599	Within Normal Limits	
C	M	80 mg/kg/day	2602	Within Normal Limits	
C	F	80 mg/kg/day	2614	Within Normal Limits	
C	F	80 mg/kg/day	2618	Within Normal Limits	
D	M	320 mg/kg/day	2596	Within Normal Limits	
D	M	320 mg/kg/day	2604	Within Normal Limits	
D	F	320 mg/kg/day	2587	Within Normal Limits	
D	F	320 mg/kg/day	2609	Within Normal Limits	
Arteether IM					
E	M	20 mg/kg/day	2597	Neuron Degeneration, hind brain	+2
E	M	20 mg/kg/day	2603	Neuron Degeneration, hind brain	+3
E	F	20 mg/kg/day	2610	Neuron Degeneration, hind brain	+4
E	F	20 mg/kg/day	2615	Neuron Degeneration, hind brain	+3

Severity Codes:

+2 = mild
+3 = moderate
+4 = severe

Table 32

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Microscopic Findings: Nonbrain Tissues

Dose (mg/kg/day)	0		320 Artelinic Acid		20 Arteether	
Animal Number	V V V V M M F F 2 2 2 2 5 5 6 6 9 9 1 1 4 8 1 2		D D D D M M F F 2 2 2 2 6 6 6 6 0 0 0 0 1 5 7 8		E E E E M M F F 2 2 2 2 5 6 6 6 9 0 1 1 7 3 5 0	
Day of Death/Sacrifice	1 2 1 2 5 1 5 1	I	1 2 0 2 5 1 4 1		1 2 1 1 5 2 5 5	I
Tissue -Lesion						
Heart -subacute inflammation, myocardium	0 1 0 0	1/4	0 0 0 0	0/4	0 0 0 0	0/4
Lung -histiocytic cellular infiltration -chronic active inflammation -hemmorhage -edema	1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	1/4 0/4 0/4 0/4	0 0 0 0 0 0 0 0 0 0 3 0 0 0 4 0	0/4 0/4 1/4 1/4	0 0 0 0 1 0 2 3 0 0 0 0 0 0 0 0	0/4 3/4 0/4 0/4
Bone marrow -hyperplasia -depletion	0 0 0 0 0 0 0 0	0/4 0/4	0 0 0 0 0 0 0 0	0/4 0/4	4 0 0 3 0 0 2 0	2/4 1/4
Thymus -lymphoid depletion	0 0 0 0	0/4	M 3 0 3	2/3	0 0 3 4	2/4
Spleen -lymphoid depletion -hematopoietic cell proliferation -reticulum cell hyperplasia	0 0 0 0 0 0 0 0 0 0 0 0	0/4 0/4 0/4	0 0 0 0 0 0 0 0 0 0 0 0	0/4 0/4 0/4	3 0 3 0 3 0 2 2 0 0 3 0	2/4 3/4 1/4
Lymph node -lymphoid depletion, bronchial -hemorrhage, bronchial -lymphoid depletion, mandibular -hemorrhage, mandibular -polymorphocellular infiltration -lymphoid depletion, mesenteric -hemorrhage, mesenteric -suppurative inflammation, iliac -hemorrhage, iliac	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 * * * * * * * *	0/4 0/4 0/4 0/4 0/4 0/4 0/4 0/0 0/0	0 0 0 0 0 0 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 * * * * * * * *	0/4 1/4 0/4 0/4 0/4 0/4 0/4 0/0 0/0	0 0 3 0 0 0 0 0 0 0 2 2 0 0 0 2 0 0 0 2 0 0 3 2 0 0 0 2 * * * 4 * * * 2	1/4 0/4 2/4 1/4 1/4 2/4 1/4 1/1 1/1
Tonsil -lymphoid depletion	0 0 0 0	0/4	0 0 0 0	0/4	0 0 3 0	1/4
Liver -hematopoietic cell proliferation -hyperplasia, Kupffer cell -diffuse necrosis, hepatocyte	0 0 0 0 0 0 0 0 0 0 0 0	0/4 0/4 0/4	0 0 0 0 0 0 0 0 0 0 1 0	0/4 0/4 1/4	3 0 3 4 0 0 2 0 0 0 0 0	3/4 1/4 0/4

0 = Lesion not observed
1 = Lesion of minimal severity
2 = Lesion of mild severity
3 = Lesion of moderate severity
4 = Lesion of marked severity
X = Lesion observed; severity not graded
M = Missing or insufficient tissue
* = Tissue not required by protocol to be examined

Table 32 (Continued)

Effect of Artelinic Acid on Dogs After Oral Administration for 14 Days

Microscopic Findings: Nonbrain Tissues

Dose (mg/kg/day)	0		320 Artelinic Acid		20 Arteether	
Animal Number	V V V V M M F F 2 2 2 2 5 5 6 6 9 9 1 1 4 8 1 2		D D D D M M F F 2 2 2 2 6 6 6 6 0 0 0 0 1 5 7 8		E E E E M M F F 2 2 2 2 5 6 6 6 9 0 1 1 7 3 5 0	
Day of Death/Sacrifice	1 2 1 2 5 1 5 1	I	1 2 0 2 5 1 4 1		1 2 1 1 5 2 5 5	I
Drug-Related Lesions						
Gallbladder -edema	0 0 0 0	0/4	0 0 0 0	0/4	0 0 3 0	1/4
Pancreas -cytoplasmic alteration, acinar cell	0 0 0 0	0/4	0 0 0 0	0/4	0 0 3 0	1/4
Large intestine -hemorrhage, mucosa, colon	0 0 0 0	0/4	0 0 0 0	0/4	0 0 0 2	1/4
Testis -hypospermia	0 0 * *	0/2	2 0 * *	1/2	0 2 * *	1/2
-cellular atypia	0 0 * *	0/2	2 0 * *	1/2	0 2 * *	1/2
Pituitary gland -cyst, pars distalis	0 0 0 0	0/4	X 0 X 0		X 0 0 0	
-cyst, pars nervosa	0 0 0 0	0/4	0 0 0 0		0 0 0 X	
Adrenal gland -congestion, cortex	M 0 0 0	0/3	0 0 4 0	1/4	0 0 0 0	0/4
-congestion, medulla	M 0 0 0	0/3	0 0 4 0	1/4	0 0 0 0	0/4
-atrophy, medulla	M 0 0 0	0/3	0 0 0 0	0/4	0 3 0 0	1/4
Sciatic nerve -subacute inflammation, epineurium	0 0 0 0	0/4	0 0 0 0	0/4	0 2 0 0	1/4
Skin -chronic active inflammation, abdomen	2 M 0 0	1/3	0 0 0 0	0/4	0 0 0 0	0/4
Skeletal muscle -hypercellularity	0 0 0 0	0/4	0 0 0 2	1/4	0 0 0 0	0/4
-chronic active inflammation	0 0 0 0	0/4	0 0 0 0	0/4	0 0 0 4	1/4
Oral mucosa -erosion	* * * *	0/0	* * * *	0/0	* * 3 0	1/2
-ulcer	* * * *	0/0	* * * *	0/0	* * 0 3	1/2

0 = Lesion not observed
1 = Lesion of minimal severity
2 = Lesion of mild severity
3 = Lesion of moderate severity
4 = Lesion of marked severity
X = Lesion observed; severity not graded
M = Missing or insufficient tissue
* = Tissue not required by protocol to be examined

Table 33

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Summary of Mortality: LD_{50} Phase, Males

Dose Group	Formulation	Dose Level (mg/kg/day)	Day of Study									Number Dead/ Number Dosed
			1	2	3	4	5	6	7	8	9	
1	AL Vehicle Control	0	0	0	0	0	0	0	0	0	a	0/2
2	AL/Lysine	80	0	0	0	0	0	0	0	0	a	0/2
3	AL/Lysine	40	0	0	0	0	0	0	0	0	a	0/2
4	AL/Lysine	20	0	0	0	0	0	0	0	0	a	0/2
5	AS Vehicle Control	0	0	0	0	0	0	0	0	0	a	0/2
6	Artesunate	400	0	2								2/2
7	Artesunate	200	0	0	0	0	0	0	0	0	a	0/2
8	Artesunate	100	0	0	0	0	0	0	0	0	a	0/2
9	Artesunate	50	0	0	0	0	0	0	0	0	a	0/2
10	AL/Lysine	320	2									2/2
11	AL/Lysine	160	0	0	0	0	0	0	0	a		0/2

[a] Scheduled sacrifice; scheduled sacrifices are not tabulated, table includes unscheduled deaths only.

Table 33 (Continued)

Summary of Mortality: LD_{50} Phase, Females

Dose Group	Formulation	Dose Level (mg/kg/day)	Day of Study									Number Dead/ Number Dosed
			1	2	3	4	5	6	7	8	9	
1	AL Vehicle Control	0	0	0	0	0	0	0	0	0	a	0/2
2	AL/Lysine	80	0	0	0	0	0	0	0	0	a	0/2
3	AL/Lysine	40	0	0	0	0	0	0	0	0	a	0/2
4	AL/Lysine	20	0	0	0	0	0	0	0	0	a	0/2
5	AS Vehicle Control	0	0	0	0	0	0	0	0	0	a	0/2
6	Artesunate	400	0	2								2/2
7	Artesunate	200	0	0	0	0	0	0	0	0	a	0/2
8	Artesunate	100	0	0	0	0	0	0	0	0	a	0/2
9	Artesunate	50	0	0	0	0	0	0	0	0	a	0/2
10	AL/Lysine	320	2									2/2
11	AL/Lysine	160	2									2/2

[a] Scheduled sacrifice; scheduled sacrifices are not tabulated, table includes unscheduled deaths only.

Table 34

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Body Weight Summary: Range Finding Phase 2, Males

Bodyweights (grams)

Group Sex		Day numbers relative to Start Date									
		-1	1	2	3	4	5	6	7	15	23
1M	Mean	240.10	246.43	256.13	262.30	268.37	275.63	284.43	287.10	332.90	373.87
	S.D.	5.31	1.76	2.59	3.90	5.77	7.88	8.98	9.81	11.97	13.75
	N	3	3	3	3	3	3	3	3	3	3
2M	Mean	239.90	253.63	260.13	264.97	268.53	269.93	276.87	280.17	333.47	374.73
	S.D.	5.96	8.35	5.04	4.59	2.97	6.26	6.14	9.17	16.56	19.19
	N	3	3	3	3	3	3	3	3	3	3
3M	Mean	239.63	250.27	254.33	260.73	265.27	270.57	274.47	277.57	321.30	360.00
	S.D.	5.16	6.21	4.17	6.12	2.18	2.63	1.55	1.31	4.12	6.30
	N	3	3	3	3	3	3	3	3	3	3
4M	Mean	239.53	246.67	253.47	259.13	266.50	273.00	278.97	286.03	334.03	379.73
	S.D.	4.58	3.76	2.25	2.03	3.40	4.07	2.00	2.97	12.20	15.44
	N	3	3	3	3	3	3	3	3	3	3
5M	Mean	239.70	248.10	257.47	261.20	268.33	272.83	279.80	281.97	328.60	367.47
	S.D.	6.95	7.05	7.31	11.22	14.19	19.10	22.75	24.16	47.35	66.69
	N	3	3	3	3	3	3	3	3	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 34 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Body Weight Summary: Range Finding Phase 2, Males

Bodyweights (grams)

Group Sex		Day numbers relative to Start Date									
		-1	1	2	3	4	5	6	7	15	23
6M	Mean	239.77	245.67	253.97	258.83	264.23	270.00	275.90	279.37	322.70	360.17
	S.D.	5.00	4.92	4.16	5.18	4.78	4.50	2.25	2.93	4.78	2.07
	N	3	3	3	3	3	3	3	3	3	3
7M	Mean	237.10	241.87	246.17	248.47	250.27	255.53	257.10	265.23	312.40	356.30
	S.D.	4.42	6.22	9.68	6.44	7.74	8.10	5.40	4.48	9.88	11.48
	N	3	3	3	3	3	3	3	3	3	3
8M	Mean	240.23	247.13	250.17	256.97	264.23	269.07	273.77	272.47	331.33	370.43
	S.D.	4.90	5.73	4.80	5.59	11.32	10.54	12.43	13.45	23.59	28.59
	N	3	3	3	3	3	3	3	3	3	3
9M	Mean	240.30	247.73	254.43	261.50	266.47	271.23	278.10	281.70	330.20	368.97
	S.D.	4.93	6.18	5.93	5.37	7.46	7.57	9.79	11.08	21.96	28.07
	N	3	3	3	3	3	3	3	3	3	3
10M	Mean	239.47	245.70	250.00	254.97	261.93	265.23	270.63	278.57	329.70	371.80
	S.D.	4.61	6.26	9.56	11.69	15.50	15.04	14.59	17.65	27.54	30.44
	N	3	3	3	3	3	3	3	3	3	3
11M	Mean	242.17	250.97	258.87	265.10	271.13	274.27	279.10	287.03	338.13	382.67
	S.D.	6.56	7.21	8.44	9.46	11.55	15.06	15.72	17.39	27.33	42.77
	N	3	3	3	3	3	3	3	3	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 34 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Body Weight Summary: Range Finding Phase 2, Females

Bodyweights (grams)

Group Sex		Day numbers relative to Start Date									
		-2	1	2	3	4	5	6	7	15	23
1F	Mean	186.70	194.73	193.37	196.80	194.10	200.73	200.70	204.50	225.83	237.17
	S.D.	3.95	5.89	7.65	3.68	1.11	4.05	7.20	4.78	7.40	7.79
	N	3	3	3	3	3	3	3	3	3	3
2F	Mean	187.77	202.07	199.77	205.33	203.80	204.83	204.60	210.80	236.93	252.17
	S.D.	4.14	3.15	6.64	1.23	0.70	2.40	3.55	3.50	0.86	6.92
	N	3	3	3	3	3	3	3	3	3	3
3F	Mean	187.83	198.50	197.17	195.57	195.93	198.73	199.77	197.10	214.13	228.60
	S.D.	8.01	6.76	6.96	4.59	1.99	1.01	2.27	1.77	4.20	4.71
	N	3	3	3	3	3	3	3	3	3	3
4F	Mean	186.63	196.60	188.20	195.37	193.30	199.13	197.47	199.30	222.90	236.60
	S.D.	7.62	8.51	7.64	14.40	10.13	11.67	10.92	16.13	26.48	32.37
	N	3	3	3	3	3	3	3	3	3	3
5F	Mean	185.87	193.13	189.93	191.00	191.47	194.43	193.13	197.10	210.87	224.07
	S.D.	5.56	5.58	0.84	6.32	9.19	9.12	2.32	5.28	5.17	11.59
	N	3	3	3	3	3	3	3	3	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day Group 2 - AL: 37.5 mg/kg/day Group 3 - AL: 18.8 mg/kg/day
Group 4 - AL: 9.7 mg/kg/day Group 5 - AL: 4.7 mg/kg/day Group 6 - AS: 0 mg/kg/day
Group 7 - AS: 75 mg/kg/day Group 8 - AS: 37.5 mg/kg/day Group 9 - AS: 18.8 mg/kg/day
Group 10 - AS: 9.4 mg/kg/day Group 11 - AE 25 mg/kg/day
Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day Group 13 - AL/Lysine: 80 mg/kg/day
Group 14 - AS Vehicle: 0 mg/kg/day Group 15 - AS: 150 mg/kg/day

Table 34 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Body Weight Summary: Range Finding Phase 2, Females

Bodyweights (grams)

Group Sex		Day numbers relative to Start Date									
		-2	1	2	3	4	5	6	7	15	23
6F	Mean	186.37	202.57	203.33	202.20	202.30	212.20	210.37	208.63	230.73	245.50
	S.D.	10.14	8.62	9.39	14.95	6.07	14.39	13.98	16.87	25.61	21.96
	N	3	3	3	3	3	3	3	3	3	3
7F	Mean	187.53	190.13	186.50*	185.37	186.53*	189.70*	193.00	194.00	213.83	227.90
	S.D.	5.54	5.86	6.06	5.25	6.93	8.91	9.31	9.84	17.06	14.34
	N	3	3	3	3	3	3	3	3	3	3
8F	Mean	184.30	190.77	191.07	193.90	195.13	201.90	201.63	201.80	225.53	239.20
	S.D.	7.10	9.08	6.96	3.45	8.16	6.54	9.54	9.87	16.78	16.00
	N	3	3	3	3	3	3	3	3	3	3
9F	Mean	186.87	195.17	193.90	191.97	192.17	197.27	198.17	198.83	216.37	226.37
	S.D.	8.21	8.27	6.93	5.26	6.88	6.16	5.17	4.21	8.09	11.06
	N	3	3	3	3	3	3	3	3	3	3
10F	Mean	190.07	197.63	201.03	203.03	205.20	206.77	206.63	209.53	224.83	234.43
	S.D.	7.47	3.89	1.11	3.36	3.55	2.95	4.36	3.61	8.22	14.18
	N	3	3	3	3	3	3	3	3	3	3
11F	Mean	186.80	191.97	192.50	195.00	194.23	194.53	195.73	196.83	215.03	228.90
	S.D.	10.28	9.81	8.72	7.58	10.47	7.82	8.06	12.34	5.18	5.70
	N	3	3	3	3	3	3	3	3	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 34 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Body Weight Summary: Range Finding Phase 3, Males

Bodyweights (grams)

Day numbers relative to Start Date

Group Sex		-1	1	2	3	4	5	6	7	8	15	23
12M	Mean	239.73	252.47	258.37	266.97	273.10	280.60	284.57	290.83	297.17	.	.
	S.D.	4.54	6.69	6.93	10.27	10.21	12.34	13.18	13.30	16.85	.	.
	N	3	3	3	3	3	3	3	3	3	0	0
13M	Mean	237.00	251.00	228.03**	223.53**	223.30**	220.53**	228.03**	234.73**	242.20**	.	.
	S.D.	4.91	4.14	8.28	9.87	6.16	10.72	10.79	9.79	6.49	.	.
	N	3	3	3	3	3	3	3	3	3	0	0
14M	Mean	238.33	251.80	259.07	264.70	272.57	277.30	280.57	286.63	.	328.33	382.03
	S.D.	4.67	4.71	2.87	2.00	3.13	3.14	5.05	5.35	.	7.51	8.88
	N	3	3	3	3	3	3	3	3	0	3	3
15M	Mean	239.80	255.33	252.03	256.80	259.90	263.57	267.97	272.77	.	321.53	364.97
	S.D.	5.37	3.52	7.72	7.67	14.18	12.21	8.95	9.68	.	6.22	9.70
	N	3	3	3	3	3	3	3	3	0	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 34 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Body Weight Summary: Range Finding Phase 3, Females

Bodyweights (grams)

Day numbers relative to Start Date

Group Sex		-1	1	2	3	4	5	6	7	8	15	23
12F	Mean	203.50	213.90	213.20	212.17	220.63	220.70	221.27	222.90	232.37	.	.
	S.D.	5.54	3.18	5.96	5.51	8.78	6.66	3.07	6.22	8.40	.	.
	N	3	3	3	3	3	3	3	3	3	0	0
13F	Mean	203.53	218.43	203.23	201.47	208.63	192.37*	180.77**	185.03*	189.73*	.	.
	S.D.	7.62	10.35	9.03	9.28	7.50	11.05	12.90	13.13	16.90	.	.
	N	3	3	3	3	3	3	3	3	3	0	0
14F	Mean	202.77	212.03	212.23	213.23	219.10	219.03	221.33	222.60	.	239.77	258.47
	S.D.	8.65	9.25	9.56	11.82	10.10	9.92	8.48	11.59	.	16.30	22.05
	N	3	3	3	3	3	3	3	3	0	3	3
15F	Mean	201.37	206.97	199.50	193.10	190.90	185.00	193.75*	195.20	.	226.75	257.00
	S.D.	8.25	7.28	13.07	14.07	17.90	23.68	10.96	13.29	.	21.43	19.80
	N	3	3	3	3	3	3	2	2	0	2	2

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 2, Males

Day: 15 relative to Start Date

Group Sex		WBC 10^3/mm3	RBC 10^6/mm3	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/mm3	Retic %	Retic Count 10^5/mm3	Neut 10^3/mm3	Lymph 10^3/mm3	Mono 10^3/mm3	Eos 10^3/mm3
1M	Mean	16.797	7.187	14.47	42.93	59.80	20.13	33.67	925.7	3.77	2.697	2.247	13.830	0.287	0.137
	S.D.	5.327	0.332	0.29	0.55	2.36	1.12	0.55	58.0	0.40	0.162	0.623	4.396	0.125	0.031
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2M	Mean	13.507	6.997	14.77	43.93	62.80	21.13	33.63	823.0	6.47	4.550	1.403	11.363	0.300	0.143
	S.D.	1.383	0.447	0.60	2.21	3.12	1.32	0.76	266.6	0.85	0.704	0.312	1.019	0.080	0.091
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
3M	Mean	11.670	6.703	14.23	42.53	63.50	21.23	33.43	866.3	5.97	4.000	1.510	9.617	0.247	0.083
	S.D.	2.279	0.240	0.25	0.80	2.08	0.67	0.06	108.3	1.02	0.580	0.815	1.379	0.055	0.058
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4M	Mean	14.640	6.613	14.03	41.63	63.13	21.30	33.73	935.3	6.13	3.983	1.950	12.123	0.213	0.103
	S.D.	2.287	0.656	0.70	2.45	3.06	1.23	0.35	290.3	1.80	0.830	0.377	2.663	0.012	0.045
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5M	Mean	14.450	7.410	15.03	44.67	60.33	20.30	33.67	987.0	4.10	3.000	1.700	11.980	0.333	0.123
	S.D.	1.430	0.469	0.35	1.60	2.15	0.92	1.10	145.3	2.03	1.396	0.580	1.252	0.119	0.032
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 2, Males

Day: 15 relative to Start Date

Group Sex		Baso 10^3/mm3	LUC 10^3/mm3
1M	Mean	0.163	0.137
	S.D.	0.104	0.055
	N	3	3
2M	Mean	0.147	0.153
	S.D.	0.047	0.060
	N	3	3
3M	Mean	0.083	0.123
	S.D.	0.035	0.059
	N	3	3
4M	Mean	0.100	0.147
	S.D.	0.017	0.025
	N	3	3
5M	Mean	0.167	0.147
	S.D.	0.006	0.031
	N	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 2, Males

Day: 15 relative to Start Date

Group Sex		WBC 10^3/mm3	RBC 10^6/mm3	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/mm3	Retic %	Retic Count 10^5/mm3	Neut 10^3/mm3	Lymph 10^3/mm3	Mono 10^3/mm3	Eos 10^3/mm3
6M	Mean	12.913	7.400	14.70	44.17	59.63	19.83	33.27	1159.3	3.50	2.600	1.487	10.817	0.197	0.097
	S.D.	2.969	0.439	1.06	3.39	1.10	0.40	0.35	107.7	0.26	0.334	0.823	2.036	0.074	0.029
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
7M	Mean	16.530	7.153	14.87	44.60	62.37	20.77	33.30	1019.0	5.13	3.667	1.657	14.317	0.263	0.050
	S.D.	3.565	0.150	0.40	1.87	1.42	0.25	0.61	108.9	0.59	0.497	0.389	3.150	0.051	0.036
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
8M	Mean	14.007	7.123	14.60	43.53	61.27	20.53	33.57	1011.3	4.97	3.517	1.527	11.773	0.323	0.067
	S.D.	2.512	0.486	0.66	1.91	3.33	1.10	0.06	51.8	0.67	0.280	0.363	2.050	0.035	0.064
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
9M	Mean	14.067	6.853	13.90	42.70	62.30	20.27	32.53	1115.0	6.23*	4.263*	1.653	11.743	0.347	0.117
	S.D.	0.235	0.233	0.95	1.74	0.69	0.64	0.87	141.7	1.57	1.000	1.112	1.118	0.120	0.133
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
10M	Mean	15.560	6.920	14.67	44.33	64.03	21.23	33.17	1102.3	5.17	3.583	1.520	13.377	0.310	0.050
	S.D.	1.354	0.026	0.40	1.42	2.25	0.55	0.42	219.6	1.02	0.683	0.435	1.283	0.132	0.035
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
11M	Mean	16.887	7.053	14.20	43.30	61.47	20.20	32.80	1057.3	6.70**	4.700**	1.567	14.477	0.383	0.097
	S.D.	3.251	0.302	0.30	1.25	1.63	0.56	0.10	172.9	0.52	0.326	0.232	3.114	0.065	0.021
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Day: 15 relative to Start Date

Group Sex	Baso 10^3/mm3	LUC 10^3/mm3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 2, Males

6M	Mean	0.140	0.177
	S.D.	0.026	0.086
	N	3	3
7M	Mean	0.127	0.123
	S.D.	0.021	0.021
	N	3	3
8M	Mean	0.140	0.173
	S.D.	0.026	0.055
	N	3	3
9M	Mean	0.073	0.133
	S.D.	0.012	0.006
	N	3	3
10M	Mean	0.130	0.167
	S.D.	0.036	0.075
	N	3	3
11M	Mean	0.170	0.203
	S.D.	0.044	0.097
	N	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day
Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day
Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day
Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day
Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day
Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 2, Females

Day: 15 relative to Start Date

Group Sex		WBC 10^3/mm3	RBC 10^6/mm3	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/mm3	Retic %	Retic Count 10^5/mm3	Neut 10^3/mm3	Lymph 10^3/mm3	Mono 10^3/mm3	Eos 10^3/mm3
1F	Mean	15.190	7.413	14.80	43.83	59.17	19.93	33.73	952.7	2.83	2.100	1.283	13.140	0.307	0.073
	S.D.	4.044	0.488	0.66	2.10	1.93	0.84	0.32	275.1	0.25	0.243	0.406	3.169	0.261	0.032
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
2F	Mean	12.977	6.910	13.70	42.30	61.17	19.83	32.40*	1203.0	4.50**	3.083**	1.113	11.447	0.167	0.060
	S.D.	2.023	0.598	1.04	3.91	0.47	0.21	0.44	174.9	0.52	0.068	0.510	1.887	0.031	0.020
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
3F	Mean	16.677	7.577	14.33	43.57	57.50	18.93	32.93	1153.3	4.23**	3.220**	1.973	13.967	0.240	0.123
	S.D.	2.100	0.183	0.40	0.93	1.41	0.38	0.31	307.4	0.06	0.098	0.693	1.506	0.072	0.021
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4F	Mean	12.447	7.207	13.83	42.20	58.57	19.20	32.80	989.7	4.53**	3.267**	1.077	10.817	0.173	0.100
	S.D.	2.448	0.178	0.46	1.06	2.75	1.06	0.36	155.2	0.45	0.250	0.290	2.605	0.025	0.026
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
5F	Mean	12.967	7.460	14.00	42.47	57.07	18.80	32.97	852.3	3.60	2.683*	1.267	11.027	0.167	0.177**
	S.D.	3.171	0.635	0.80	2.16	2.08	0.72	0.65	107.2	0.17	0.330	0.699	2.537	0.058	0.025
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 2, Females

Day: 15 relative to Start Date

Group Sex		Baso 10^3/mm3	LUC 10^3/mm3
1F	Mean	0.150	0.233
	S.D.	0.095	0.186
	N	3	3
2F	Mean	0.093	0.090
	S.D.	0.051	0.056
	N	3	3
3F	Mean	0.157	0.217
	S.D.	0.015	0.006
	N	3	3
4F	Mean	0.123	0.153
	S.D.	0.029	0.076
	N	3	3
5F	Mean	0.130	0.210
	S.D.	0.010	0.140
	N	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 2, Females

Day: 15 relative to Start Date

Group Sex		WBC 10^3/mm3	RBC 10^6/mm3	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/mm3	Retic %	Retic Count 10^5/mm3	Neut 10^3/mm3	Lymph 10^3/mm3	Mono 10^3/mm3	Eos 10^3/mm3
6F	Mean	14.593	7.253	14.30	43.20	59.57	19.73	33.10	999.7	3.73	2.730	1.797	12.050	0.317'	0.093
	S.D.	5.902	0.307	0.26	0.89	1.81	0.42	0.61	174.3	0.91	0.737	0.527	5.134	0.181	0.040
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
7F	Mean	15.780	7.190	13.80	41.97	58.40	19.20	32.83	924.7	5.23	3.797	3.760	11.343	0.193	0.180
	S.D.	4.504	0.355	0.66	1.68	2.55	0.61	0.50	131.1	2.46	1.869	1.837	2.597	0.064	0.252
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
8F	Mean	16.140	6.990	13.83	42.10	60.20	19.77	32.80	1099.7	5.03	3.527	1.270	14.170	0.247	0.143
	S.D.	3.597	0.078	0.50	1.51	2.10	0.76	0.10	154.4	1.53	1.029	0.252	3.644	0.035	0.038
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
9F	Mean	9.780	7.280	14.27	42.77	58.70	19.57	33.33	754.7	4.80	3.497	1.513	7.777	0.133	0.117
	S.D.	1.969	0.451	0.85	2.66	0.00	0.12	0.15	246.1	0.52	0.220	0.731	2.090	0.057	0.055
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
10F	Mean	13.237	7.273	14.17	42.37	58.33	19.50	33.43	924.3	3.07	2.237	1.190	11.530	0.147	0.090
	S.D.	4.855	0.434	0.25	0.64	2.80	0.85	0.32	30.9	0.23	0.188	0.439	4.687	0.072	0.026
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
11F	Mean	13.523	7.137	13.77	42.17	59.10	19.30	32.70	1028.7	5.00	3.553	1.123	11.737	0.297	0.137
	S.D.	1.594	0.343	0.67	1.88	0.40	0.10	0.17	103.5	0.20	0.071	0.301	1.742	0.162	0.055
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 2, Females

Day: 15 relative to Start Date

Group Sex		Baso 10^3/mm3	LUC 10^3/mm3
6F	Mean	0.093	0.137
	S.D.	0.023	0.074
	N	3	3
7F	Mean	0.123	0.180
	S.D.	0.040	0.026
	N	3	3
8F	Mean	0.127	0.183
	S.D.	0.025	0.040
	N	3	3
9F	Mean	0.080	0.160
	S.D.	0.010	0.182
	N	3	3
10F	Mean	0.127	0.150
	S.D.	0.070	0.089
	N	3	3
11F	Mean	0.103	0.123
	S.D.	0.040	0.012
	N	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 3, Males

Day: 8 relative to Start Date

Group Sex		WBC 10^3/mm3	RBC 10^6/mm3	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/mm3	Retic %	Retic Count 10^5/mm3	Neut 10^3/mm3	Lymph 10^3/mm3	Mono 10^3/mm3	Eos 10^3/mm3
12M	Mean	16.987	7.033	13.63	41.70	59.37	19.40	32.67	1063.0	4.50	3.167	2.820	13.520	0.287	0.103
	S.D.	1.405	0.172	0.32	1.25	3.04	0.95	0.45	209.7	0.95	0.722	0.589	1.568	0.081	0.081
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
13M	Mean	33.270	5.880	11.13	34.57	58.83	19.00	32.23	1124.0	7.33	4.390	10.787	21.083	0.653	0.163
	S.D.	7.845	0.633	1.11	3.58	0.93	0.52	0.42	513.9	2.41	1.873	4.333	4.999	0.116	0.127
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Group Sex		Baso 10^3/mm3	LUC 10^3/mm3
12M	Mean	0.070	0.183
	S.D.	0.010	0.040
	N	3	3
13M	Mean	0.203	0.377
	S.D.	0.050	0.170
	N	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 3, Males

Day: 15 relative to Start Date

Group Sex		WBC 10^3/mm3	RBC 10^6/mm3	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/mm3	Retic %	Retic Count 10^5/mm3	Neut 10^3/mm3	Lymph 10^3/mm3	Mono 10^3/mm3	Eos 10^3/mm3
14M	Mean	16.400	7.403	15.37	45.37	61.27	20.73	33.83	1288.7	3.47	2.553	2.230	13.623	0.237	0.063
	S.D.	1.521	0.127	0.25	0.76	1.15	0.51	0.21	337.3	0.38	0.275	0.895	1.262	0.101	0.031
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
15M	Mean	18.323	7.400	15.47	46.47	62.80	20.87	33.23	1013.7	5.07	3.757	2.857	14.853	0.283	0.050
	S.D.	0.359	0.235	0.76	2.02	1.35	0.38	0.60	122.0	0.83	0.522	0.692	0.711	0.083	0.026
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Group Sex		Baso 10^3/mm3	LUC 10^3/mm3
14M	Mean	0.073	0.177
	S.D.	0.023	0.099
	N	3	3
15M	Mean	0.100	0.177
	S.D.	0.035	0.072
	N	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 3, Females

Day: 8 relative to Start Date

Group Sex		WBC 10^3/mm3	RBC 10^6/mm3	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/mm3	Retic %	Retic Count 10^5/mm3	Neut 10^3/mm3	Lymph 10^3/mm3	Mono 10^3/mm3	Eos 10^3/mm3
12F	Mean	15.593	7.253	13.73	41.07	56.67	18.90	33.37	1062.7	3.50	2.553	1.647	13.427	0.197	0.113
	S.D.	3.026	0.226	0.21	0.90	2.24	0.35	0.74	205.9	0.10	0.116	0.488	2.490	0.032	0.025
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
13F	Mean	32.423	5.720	11.07	32.50	57.10	19.40	33.97	2058.7	3.03	1.490	8.460	22.240	0.920	0.193
	S.D.	5.868	1.033	1.92	4.98	2.65	0.78	0.70	239.5	3.80	1.629	4.681	4.683	0.234	0.223
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3

Group Sex		Baso 10^3/mm3	LUC 10^3/mm3
12F	Mean	0.073	0.137
	S.D.	0.025	0.035
	N	3	3
13F	Mean	0.163	0.447
	S.D.	0.065	0.150
	N	3	3

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

Table 35 (Continued)

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats

Hematology Summary Report: Range Finding Phase 3, Females

Day: 15 relative to Start Date

Group Sex		WBC 10^3/mm3	RBC 10^6/mm3	HGB g/dL	HCT %	MCV fL	MCH pg	MCHC g/dL	PLT 10^3/mm3	Retic %	Retic Count 10^5/mm3	Neut 10^3/mm3	Lymph 10^3/mm3	Mono 10^3/mm3	Eos 10^3/mm3
14F	Mean	18.483	7.820	15.80	45.77	58.50	20.23	34.57	1328.3	3.13	2.437	2.027	15.883	0.190	0.060
	S.D.	5.990	0.156	0.17	0.45	0.80	0.35	0.12	305.9	0.21	0.206	0.909	4.854	0.010	0.036
	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3
15F	Mean	21.295	6.215	13.10	39.40	63.40	21.15	33.35	1100.0	13.45	8.295	7.660	12.745	0.525	0.025
	S.D.	9.822	0.247	0.14	0.71	3.54	1.06	0.21	35.4	4.88	2.708	8.768	0.417	0.460	0.021
	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2

Group Sex		Baso 10^3/mm3	LUC 10^3/mm3
14F	Mean	0.093	0.233
	S.D.	0.059	0.129
	N	3	3
15F	Mean	0.095	0.245
	S.D.	0.021	0.177
	N	2	2

Statistical Analysis (Dunnett's): * = p < 0.05; ** = p < 0.01; treated groups were compared to the appropriate control group (i.e., Groups 2-5 were compared to Group 1, Groups 7-10 to Group 6, Group 13 was compared to Group 12, and Group 15 to Group 14).

Nominal Dose: Phase 2: Group 1 - AL: 0 mg/kg/day; Group 2 - AL: 37.5 mg/kg/day; Group 3 - AL: 18.8 mg/kg/day; Group 4 - AL: 9.7 mg/kg/day; Group 5 - AL: 4.7 mg/kg/day; Group 6 - AS: 0 mg/kg/day; Group 7 - AS: 75 mg/kg/day; Group 8 - AS: 37.5 mg/kg/day; Group 9 - AS: 18.8 mg/kg/day; Group 10 - AS: 9.4 mg/kg/day; Group 11 - AE 25 mg/kg/day

Phase 3: Group 12 - AL Vehicle: 0 mg/kg/day; Group 13 - AL/Lysine: 80 mg/kg/day; Group 14 - AS Vehicle: 0 mg/kg/day; Group 15 - AS: 150 mg/kg/day

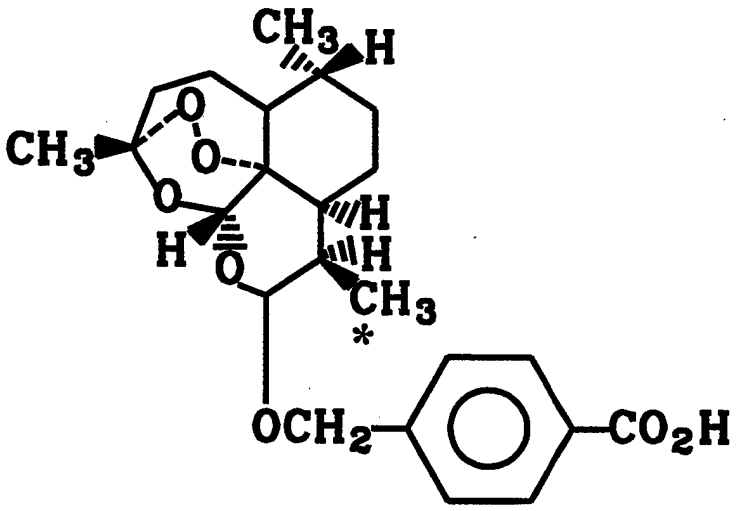

Figure 1

Structure of artelinic acid. The position of the [^{14}C] is indicated (*).

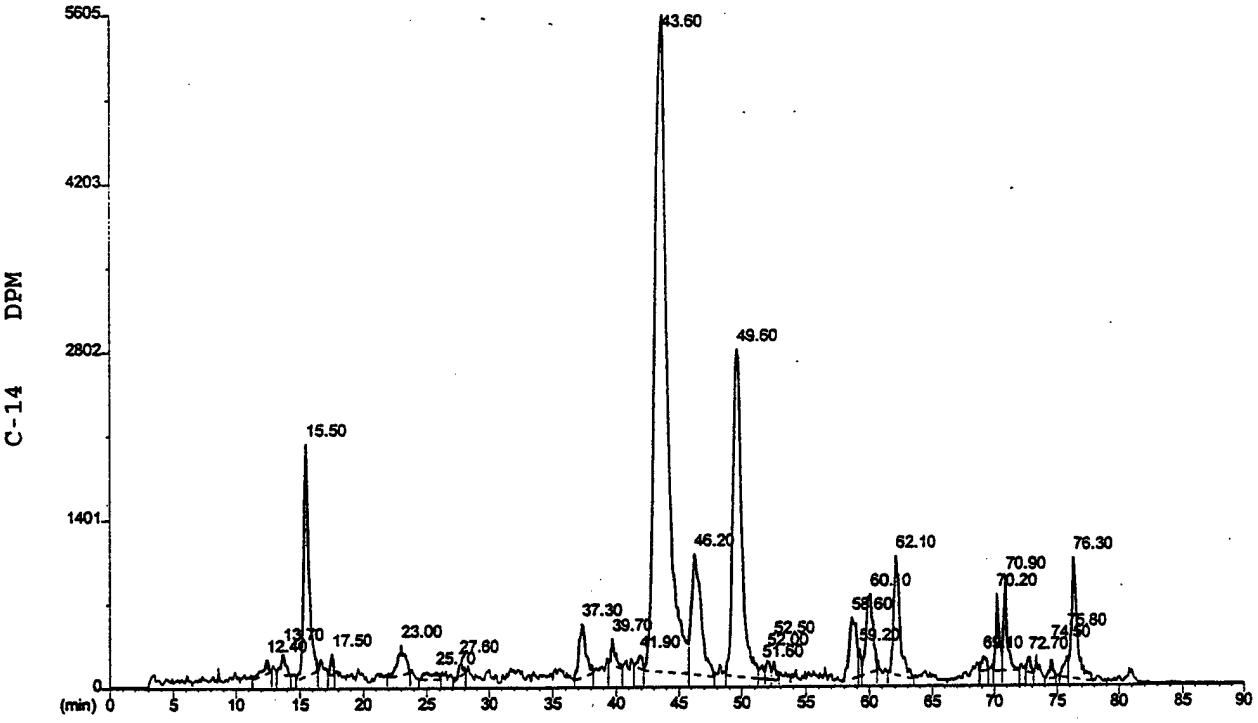

Figure 2

Representative radiochromatogram showing the radioactivity profile obtained during the HPLC analysis of urine collected 0-12 hours after IV administration of [^{14}C]artelinic acid to a dog. In this system, unchanged artelinic acid had a retention time of approximately 85 minutes.

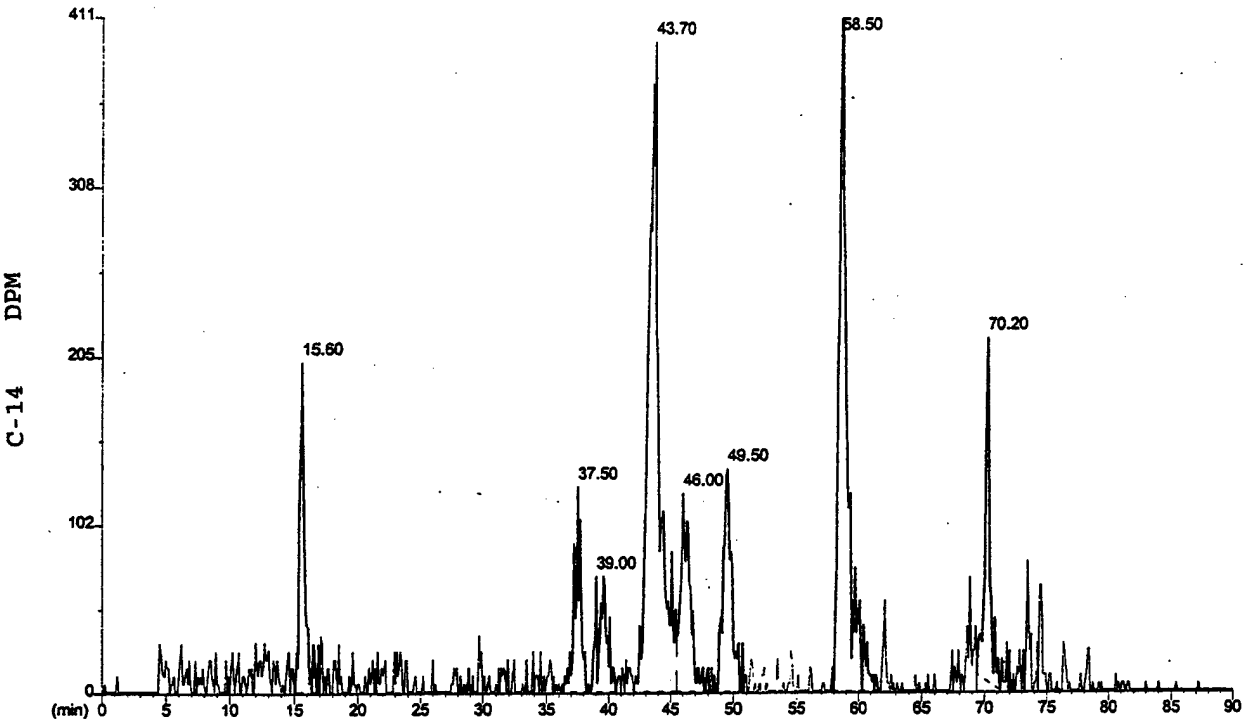

Figure 3

Representative radiochromatogram showing the radioactivity profile obtained during the HPLC analysis of urine collected 12-24 hours after IV administration of [^{14}C]artelinic acid to a dog. In this system, unchanged artelinic acid had a retention time of approximately 85 minutes.

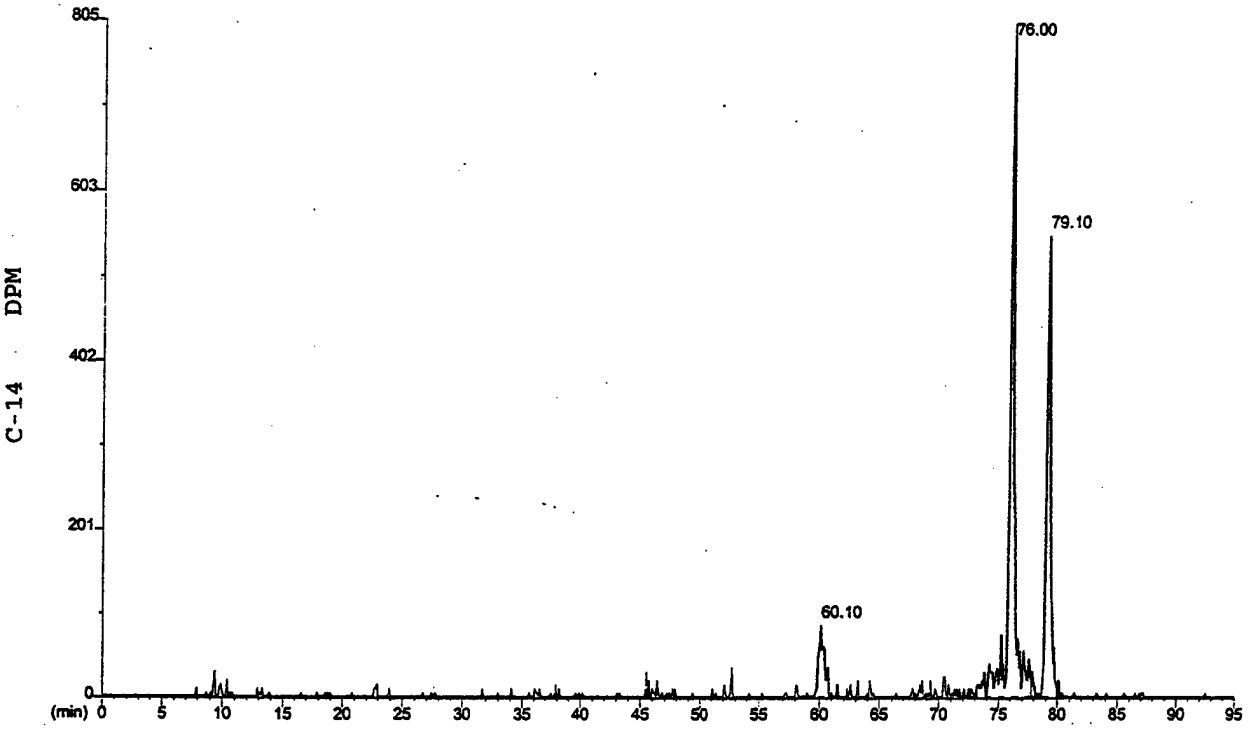

Figure 4

Representative radiochromatogram showing the radioactivity profile obtained during the HPLC analysis of feces collected 0-12 hours after IV administration of [^{14}C]artelinic acid to a dog. In this system, unchanged artelinic acid had a retention time of approximately 85 minutes.

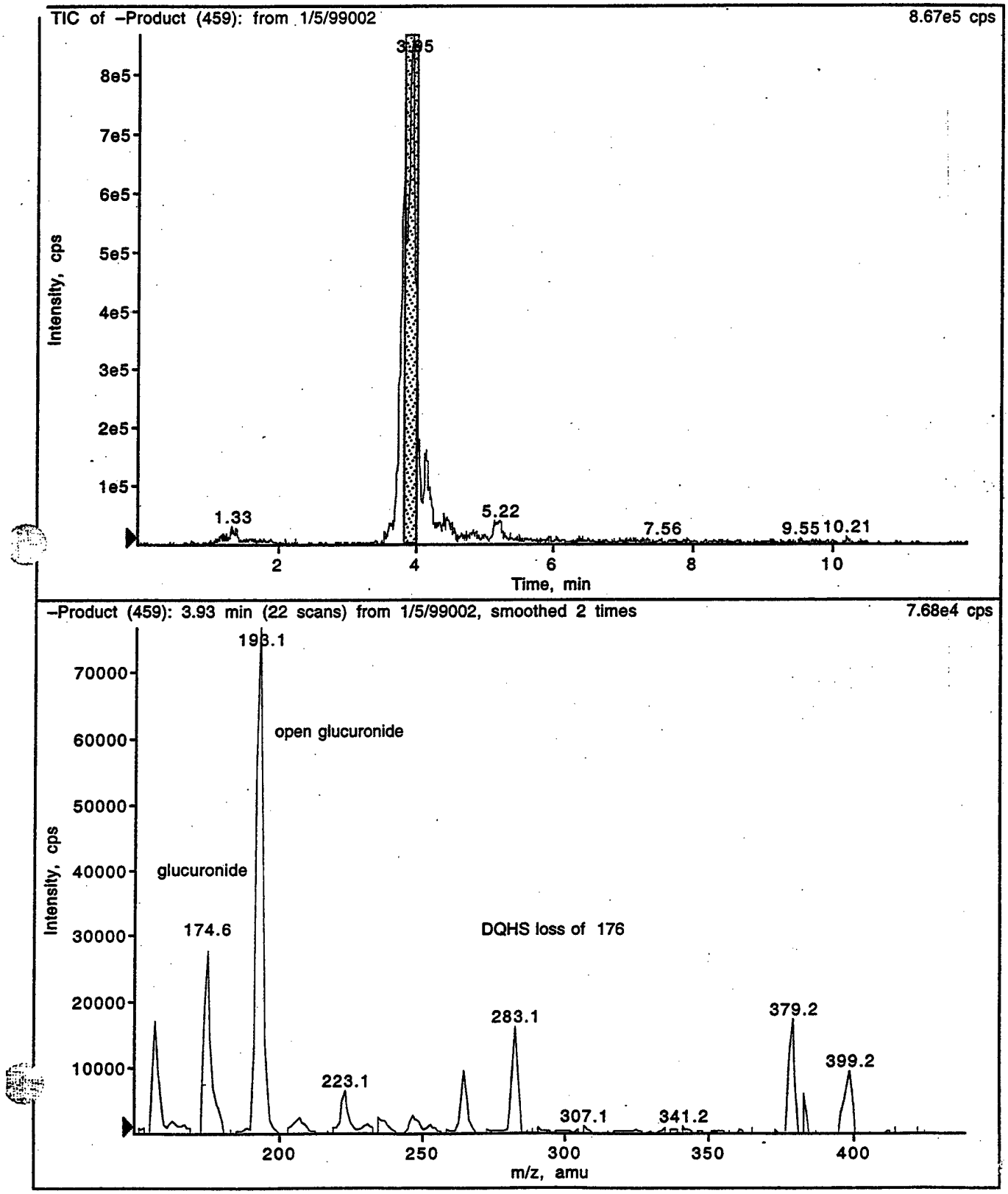

Figure 5

LC/MS/MS selected ion chromatogram of urine collected from a dog given artelinic acid (top panel) and the mass spectrum of the metabolite tentatively identified as DQHS glucuronide (bottom panel).

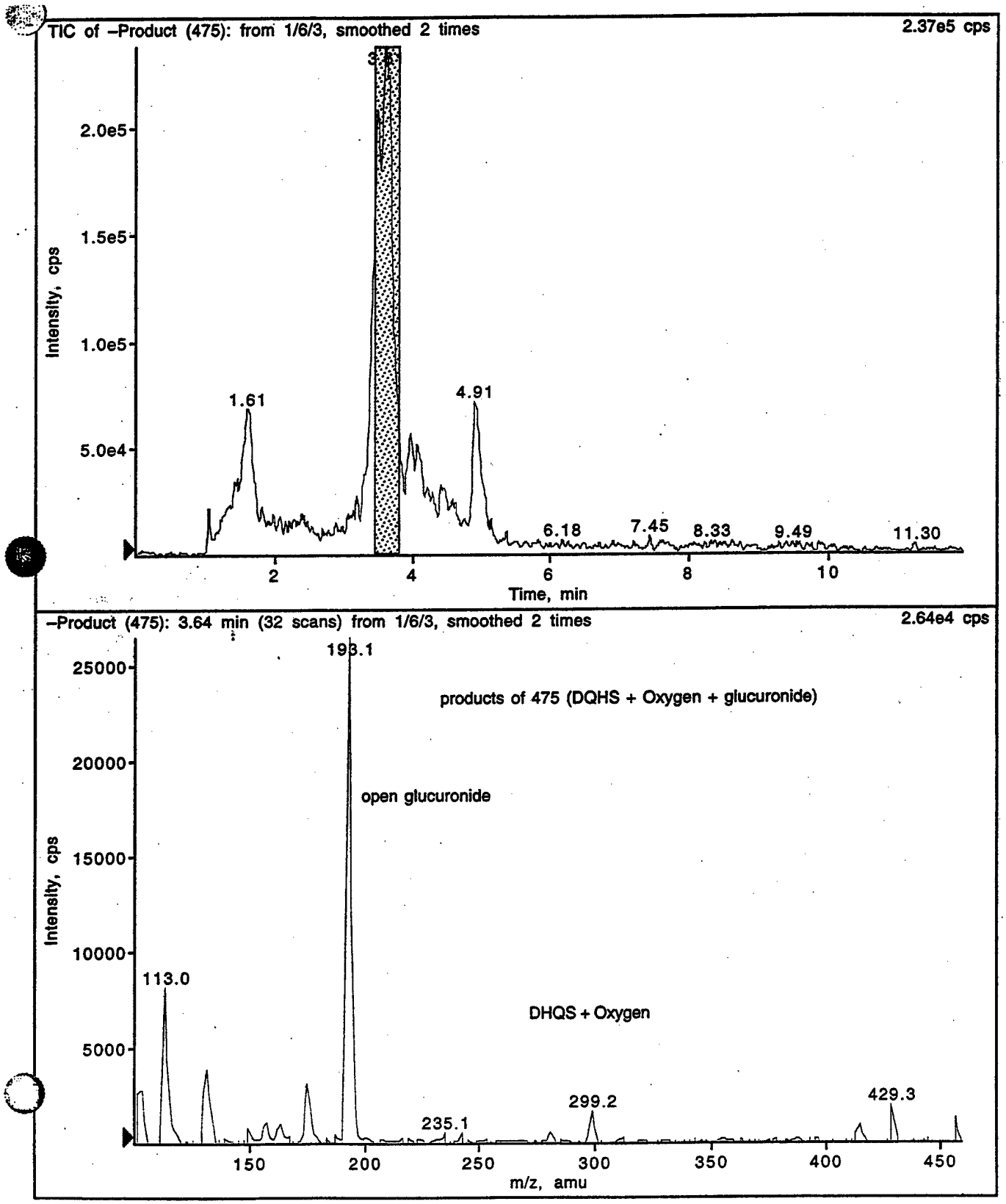

Figure 6

LC/MS/MS selected ion chromatogram of urine collected from a dog given artelinic acid (top panel) and the mass spectrum of the metabolite tentatively identified as a glucuronide of hydroxy DQHS (bottom panel).

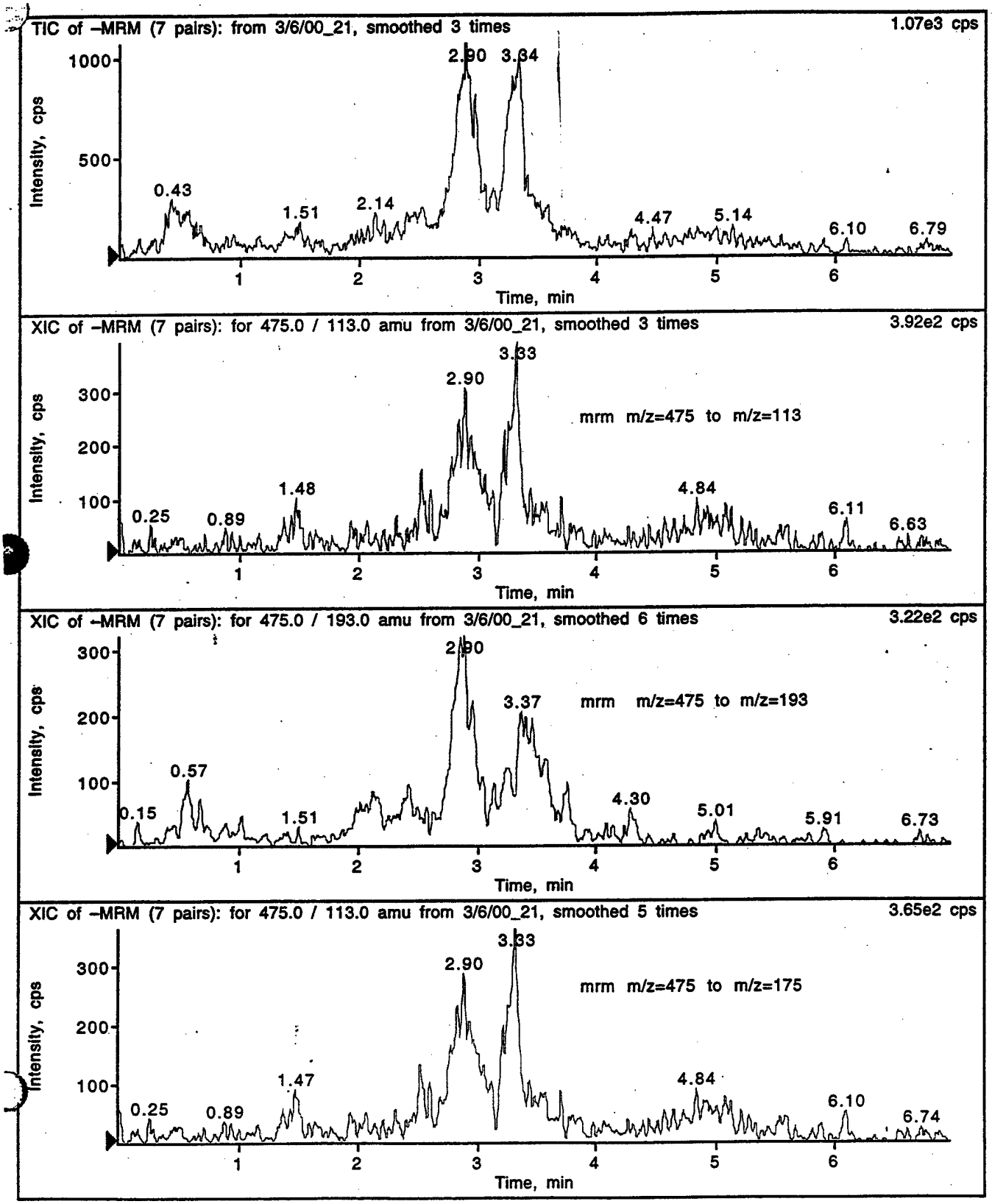

Figure 7

LC/MS/MS chromatogram of the urinary metabolite of artelinic acid
Tentatively identified as a glucuronide of hydroxy DQHS.

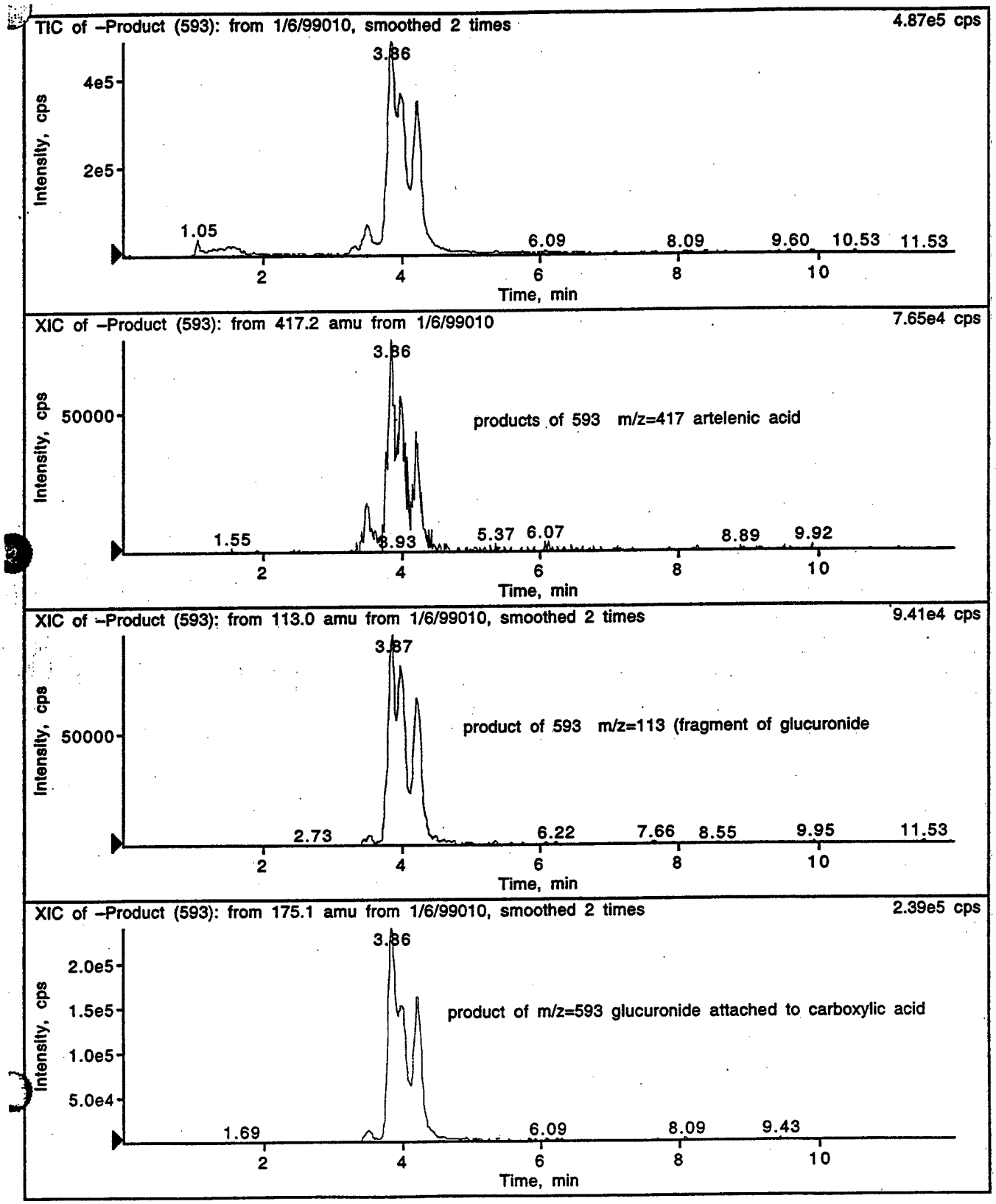

Figure 8

LC/MS/MS chromatogram of the urinary metabolite of artelinic acid tentatively identified as a glucuronide of artelinic acid.

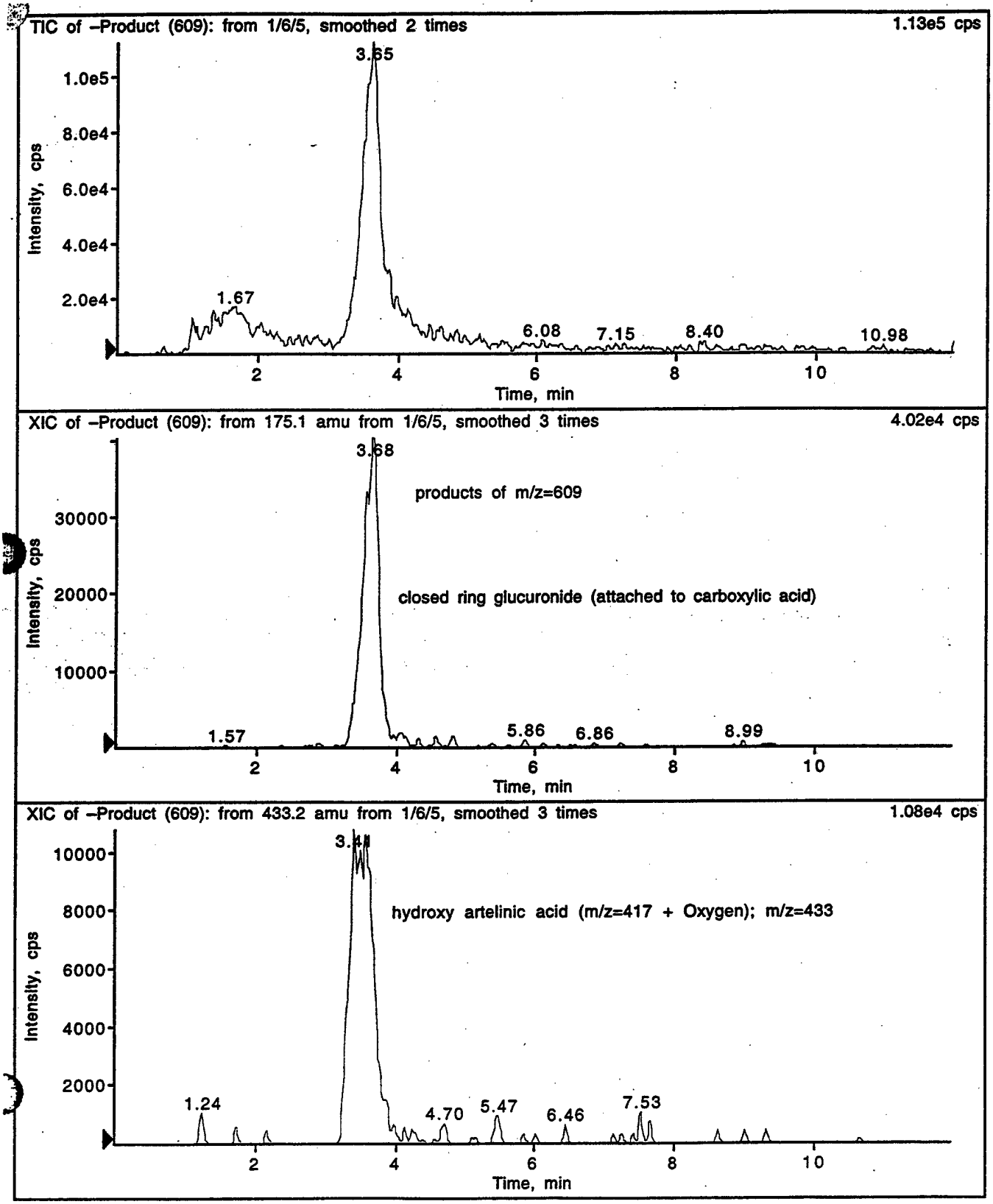

Figure 9

LC/MS/MS chromatogram of the urinary metabolite of artelinic acid tentatively identified as a glucuronide of hydroxy artelinic acid.

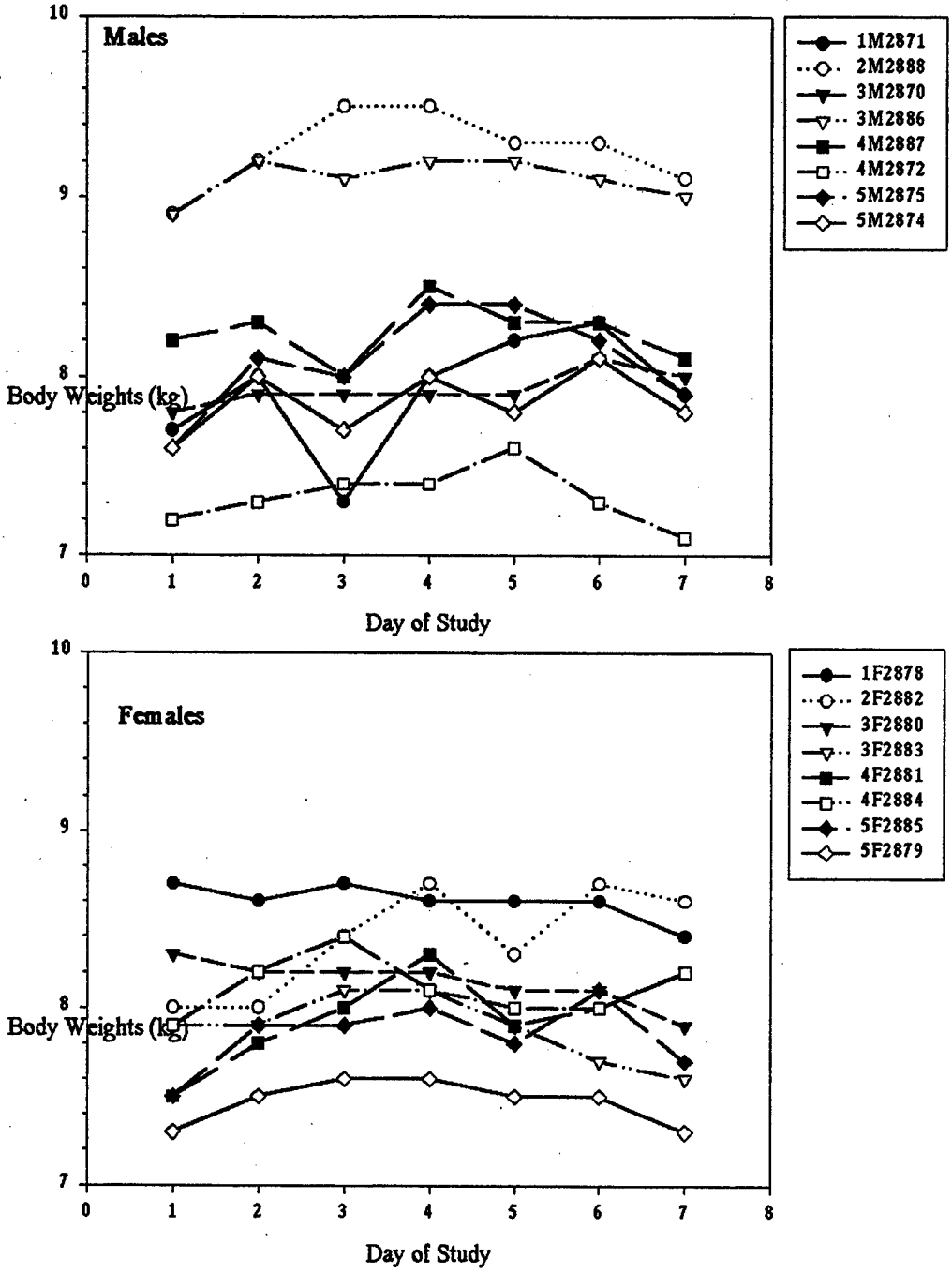

Figure 10

Study of Injectable Artelinic Acid in Dogs

Body Weights

Appendix A

Contract Personnel

Date: 02/03/04
Time: 9:35:06 AM

SOUTHERN RESEARCH INSTITUTE

Project Labor Summary

By Project, Employee/Vendor Detail
As of Fiscal Year: 2003 Period: 12 Subperiod: 1 Ending: 12/31/2003

Project:	09610	**Contract Value:**	##
Name:	USAMRAA	**Funded Value:**	##
Owning Organization:	1.03.01.08.01	**Project Classification:**	DI
Prime Contract Number:	DAMD17-98-D-0032	**Project Type:**	CP
Subcontract Number:	MOD P80001	**Period of Performance:**	09
Purchase Order:		**Active:**	Ye
Project Manager:			
Customer:			

Labor Category		Period Actual Hours	YTD Actual Hours	ITD Actual Hours
10511	MCDUFFEE, TRACI S.	0.00	0.00	24.50
10936	HENDRICKS, MICHAEL	0.00	0.00	20.50
11151	BELL, DEBRA J.	0.00	0.00	18.50
11487	EDWARDS, MARTHA M.	0.00	64.50	109.50
11576	GRIMES, MARSHA L.	0.00	0.00	29.00
11843	HARRIS, LAQUITTA R.	0.00	0.00	27.00
12068	LIN, TSU-HAN	0.00	0.00	149.00
12122	BROWN, JULIA M.	0.00	0.00	9.00
12203	THOMAS, MICHELLE	0.00	0.00	99.50
12718	WIGGINS, JESSIE M.	0.00	0.00	19.50
13609	KING, MARILYN D.	0.00	0.00	104.50
13625	MAY, DONALD W.	0.00	0.00	11.00
14117	PARSONS, DAVID R.	0.00	0.00	19.50
14192	FINLEY, WALTER	0.00	0.00	17.50
14231	MASON, MONICA W.	0.00	0.00	31.50
14281	OLIVER, CAROLYN R.	0.00	0.00	18.00
16497	ANDERSON, JAMES A.	0.00	0.00	92.00
16641	WILLIAMS, LESTER M.	0.00	0.00	24.00
16811	JOHNSON, RODNEY K.	0.00	0.00	2.00
17132	FULTON, RONNA	2.00	7.00	74.20
17230	STRIBLING, ANDREW M.	0.00	0.00	25.00
17388	WEAVER, PAMELA S.	0.00	0.00	12.00
17787	JOHNSON, SABRINA L.	0.00	0.00	11.00
17914	BELL, LISA C.	0.00	0.00	5.00
17973	MULLEN, KATHLEEN	0.00	0.00	40.00
18619	BOLING, DWAYNE K.	0.00	0.00	23.50
18627	DICAS, MARIAN C.	0.00	0.00	9.00
18635	POOLE, TERRITA L.	0.00	0.00	98.00
18864	ROBERTSON, CECILIA R.	0.00	0.00	15.00
18937	HORNE, BRETT G.	0.00	0.00	24.50
19313	BELL, JAMES D.	0.00	0.00	32.50
19321	SLATER, BYRON K.	0.00	0.00	3.50
19496	HYATT, TANYA C.	0.00	0.00	11.50
19500	WILSON, RACHEL D.	0.00	0.00	48.50
19526	RICHTER, WARD R.	0.00	0.00	176.00
19569	JOHNSON, JAMES D.	0.00	0.00	49.00
19585	HARRISON, GAIL B.	0.00	0.00	9.00
19828	WILLIAMS, JASON L.	0.00	0.00	101.00
19933	JACKSON, JOY G.	0.00	0.00	0.50
20044	BRYANT, VANESSA	0.00	0.00	6.00
20052	DAWKINS, MINDI S.	0.00	0.00	7.00
20214	GIRTMAN, MIA L.	0.00	0.00	84.00
20362	SULLEN, MARISSA G.	0.00	0.00	38.00
20524	MARSH, CATHERINE L.	0.00	0.00	6.50
20583	RISNER, ANGELA B.	0.00	0.00	31.00
20788	KIDD, SYNETHIA T.	0.00	0.00	1.50
21105	CHRISTIAN, JOYCE K.	0.00	0.00	7.70
21130	GORMAN, GREGORY S.	0.00	0.00	59.00
21199	CHAMPIGNY, VANESSA O.	0.00	0.00	27.00
21377	COUCH, RAYMOND L.	0.00	0.00	108.50
21504	GEORGE, MARVELLA C.	0.00	0.00	7.50
21610	BURDEN, LARHONDA M.	0.00	0.00	17.50
21687	STEVENSON, TAMARA	0.00	0.00	69.00
21709	SELLERS, MICHELE R.	0.00	0.00	27.50
21733	PAYNE, PARRISH L.	0.00	0.00	28.00
21938	DOLLAR, GEORGE R.	0.00	0.00	292.00
21946	BOYER, REBECCA K.	0.00	0.00	37.00

A-2

Date: 02/03/04
Time: 9:35:07 AM

SOUTHERN RESEARCH INSTITUTE

Project Labor Summary

By Project, Employee/Vendor Detail
As of Fiscal Year: 2003 Period: 12 Subperiod: 1 Ending: 12/31/2003

Project:	09610	**Contract Value:**	##
Name:	USAMRAA	**Funded Value:**	##
Owning Organization:	1.03.01.08.01	**Project Classification:**	DI
Prime Contract Number:	DAMD17-98-D-0032	**Project Type:**	CP
Subcontract Number:	MOD P80001	**Period of Performance:**	09
Purchase Order:		**Active:**	Ye
Project Manager:			
Customer:			

	Labor Category	Period Actual Hours	YTD Actual Hours	ITD Actual Hours
21954	MALPHRUS, ELIZABETH A.	0.00	0.00	5.00
21962	SKILLMAN, DANA L.	0.00	0.00	6.50
22039	BLEVINS, BRIDGETTE Y.	0.00	0.00	2.00
22098	TARPLEY, SHERRI A.	0.00	0.00	34.50
22144	CARTER, ROBIN T.	0.00	0.00	60.50
22152	LAGORY, BRIAN E.	0.00	0.00	7.00
22357	BENTON-STRICKLAND, NATERI	0.00	0.00	1.00
22497	DAVIS,JR, JIMMY W.	0.00	0.00	40.50
22551	MOON, LA'WANDA	0.00	0.00	28.00
22560	PHILLIPS, ANDREA	0.00	0.00	36.00
22659	STOWE, ELIZABETH A.	0.00	0.00	2.00
22764	DURBIN, LAJUANA A.	0.00	0.00	13.00
22829	RUGGS, SHERONE V.	0.00	0.00	0.50
22870	OFFORD, SHONNITI A	0.00	0.00	14.50
22888	SCOGGINS, JEFFERY W.	0.00	0.00	27.50
23027	GATES, JENNIFER B.	0.00	0.00	1.50
23272	ROWE, TANEAKA A.	0.00	0.00	1.00
23302	WESTRY-LEWIS, SHARELL L.	0.00	0.00	43.00
23485	HEWITT-STEENBERG, LAVINIA	0.00	0.00	61.50
23574	CALDWELL, LEE M.	0.00	0.00	5.00
24031	PICKETT, APRIL C.	0.00	0.00	1.50
25089	MORROW, LORI F.	0.00	1.50	1.50
26239	NOKER, PATRICIA E.	1.50	17.90	581.00
28517	TILLERY, KATHLEEN F.	0.00	0.00	252.50
30902	BELZER, JOAN B.	0.00	0.00	1.50
30937	HOGAN, DONNA B.	0.00	6.50	121.10
31640	CURREN, KEITH R.	0.00	0.00	84.50
32867	FOLSE, DIANE D.	0.00	0.00	162.00
33065	GARST, PHILIP H.	0.00	0.00	40.50
33073	GARRETT, AGATHER G.	0.00	0.00	25.50
33120	GILES, HERSCHELL D.	0.00	0.00	9.50
33162	GORDON, CYNTHIA D.	0.00	0.00	260.50
33537	HEATH, JAMES E.	0.00	0.00	2.00
33553	HILDRETH, EDWARD A.	0.00	0.00	18.50
34991	KING, ANTHONY	0.00	0.00	13.50
35548	MCDONOUGH, DAVID S.	0.00	0.00	25.00
35734	MARRISETTE, DORA	0.00	0.00	4.50
35769	JOHNSON, MELODY M.	0.00	0.00	22.50
35980	MOODY, ALBERT	0.00	0.00	2.00
37401	REARDON, VERONICA J.	0.00	0.00	111.50
38482	THIGPEN, FRANK A.	0.00	0.00	18.50
39276	SMITH, BRENDA W.	0.00	0.00	34.00
39691	CARPENTER, SUSAN B.	0.00	0.00	179.00
42269	EMORY, MARY B.	0.00	0.00	91.00
74250	ISBELL, CONSTANCE M.	0.00	0.00	2.00
79260	WEINBERG, DAVID S.	0.00	0.00	46.50
80926	BILLINGSLEY, ANN B.	0.00	0.00	1.00
Total for 09610:		3.50	97.40	4953.00

Appendix B

Analytical Method for the Analysis of Artelinic Acid in Dog Plasma

ANALYTICAL METHOD

Method No.: BACG-3495

Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

1.0 PRINCIPLE

Plasma samples are obtained from animals treated with artelinic acid. Plasma (e.g., 500 µL) containing artelinic acid is fortified with internal standard (IS), artesunic acid. Plasma samples and spiked standards are then mixed with acetonitrile, vortexed and centrifuged. The supernatant is removed, concentrated to dryness, reconstituted in mobile phase, filtered, and then analyzed by HPLC/ MS.

2.0 REAGENTS AND SOLUTIONS

The listed reagents or their equivalents may be used.

2.1 Neat Reagents

2.1.1 Water, deionized, organic free, from in-house system (DI water)

2.1.2 Artelinic acid, as provided by the client.

2.1.3 Artesunic acid (internal standard) as provided by client.

2.1.4 Blank control dog plasma (e.g, obtained from blood collected in tubes containing the anticoagulant ethylenediaminetetraacetic acid).

2.1.5 Methanol, high-performance liquid chromatography (HPLC) grade.

2.1.6 Formic acid (min. 88%).

2.1.7 Acetonitrile, high-performance liquid chromatography (HPLC) grade.

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Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

2.2 **Prepared Solutions**

Appropriate changes in the solutions may be made at the discretion of the analyst.

2.2.1 0.2% Formic acid in Methanol

For example, to prepare 1 l, combine formic acid (e.g. 2 mL) and methanol (e.g. 998 mL). Mix well. Filter through a 0.45-µm filter before use on the HPLC.

2.2.2 Mobile phase: 0.2% Formic acid in Methanol : water (80:20)

For example, to prepare 1 l, combine 0.2% formic acid in methanol (e.g. 800 mL) and DI water (e.g., 200 mL). Mix well. Note: This is to be used for reconstitution of samples prior to analysis.

3.0 **INSTRUMENTS, MATERIALS, AND APPARATUS**

The following or their equivalents may be used.

3.1 HPLC pump(s), autosampler, and single quadrupole mass spectrometer.

3.2 Autosampler vials.

3.3 Vortex mixer (e.g., touch mixer or IKA-Vibrax ® platform mixer).

3.4 Glass culture tubes with Teflon-lined caps.

3.5 HPLC mobile phase filtration apparatus.

3.6 Filters for HPLC mobile phase filtration apparatus (e.g., Nylon-66, 0.45 µm).

ANALYTICAL METHOD

Method No.: BACG-3495

Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

3.7 Aquasil C18 HPLC column (250 mm x 2 mm) Keystone Scientific (Cat. No. 255-775--2)

3.8 Analytical balance, readable to 5 decimal places.

3.9 Assorted pipettes, glass and plastic syringes, and glassware.

3.10 Volumetric flasks (e.g., Class A 10 mL, 25 mL, and 2 l).

3.11 Micropipettor(s).

3.12 Centrifuge.

3.13 Solvent-concentration apparatus (e.g., Zymark Turbo-Vap® with a source of nitrogen).

3.14 Ultrasonic bath.

3.15 Filtration units for standards and samples, (e.g., 0.2 μm PVDF filters and disposable syringes).

4.0 PREPARATION OF STOCKS AND WORKING STOCKS

If desired, a modified dilution scheme can be used and documented in the study records. Appropriate changes in the concentrations of the solutions may be made at the discretion of the analyst.

4.1 Stock Solution of Test Article (artelinic acid), 500 µg/mL

4.1.1 Prepare an ~500 µg/mL solution of artelinic acid in acetonitrile (e.g., accurately weigh about 10.00 mg artelinic acid into a 20-mL vial). Add acetonitrile (e.g. 20 mL) to dissolve. Alternatively, weigh the compound into a an appropriate vessel (e.g.,

ANALYTICAL METHOD

Method No.: BACG-3495

Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

culture tube) and add 10 mL of DI water. Mix well. Transfer the solution to a clean vessel if desired.

4.2 **Dilute Solution of Test Article (Artelinic Acid), ~5 µg/mL**

4.2.1 Combine the main artelinic acid stock solution (~500 µg/mL; e.g., 100 µL) with acetonitrile (e.g, 9.90 mL) or alternatively dilute to 10 mL in a 10-mL volumetric flask. Mix well. Transfer the solution to a clean vessel if desired.

4.3 **Working Stock Solutions of the Test Article**

4.3.1 To prepare working stock solutions, make the proper dilutions using acetonitrile as shown in the following table. Prepare in 10-mL volumetric flasks or in culture tubes. Store refrigerated when not in use.

Working Stock Level (WSL)	Volume of Specified Test Article Stock or WS	Final Volume in acetonitrile (mL)	Approximate Concentration (ng/mL)
WSA	~5 µg/mL dilute stock	--	5000
WSB	5 mL of WSA	10	2500
WSC	4 mL of WSB	10	1000
WSD	5 mL of WSC	10	500

4.4 **Working Stock and Dilute Stock of Internal Standard (Artesunic Acid)**

4.4.1 Prepare a solution of artesunic acid (~1 mg/mL) by weighing out approximately 10 mg of artesunic acid into a 10 mL volumetric flask and dilute to volume with acetonitrile. Mix well to ensure complete dissolution of the artesunic acid.

4.4.2 Dilute 200 µL of the ~1 mg/mL solution to 10 mL in a volumetric flask with acetonitrile and mix well. The resulting concentration of this solution is ~ 20 µg/mL

ANALYTICAL METHOD

Method No.: BACG-3495

Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

4.4.3 Accurately pipet 4 mL of the ~20 µg/mL working solution into a 10 mL volumetric flask and dilute to volume with acetonitrile. The resulting concentration of the spiking solution is ~8 µg/mL

5.0 PREPARATION OF SPIKED STANDARDS AND BLANKS

Appropriate changes in the concentrations of the standards may be made at the discretion of the analyst.

5.1 Multiple (e.g., at least five) plasma standards and one plasma blank + IS and, optionally, one plasma blank - IS are analyzed with each set of unknown samples.

5.2 Into individual culture tubes, pipette blank plasma (e.g., 500 µL). Add to each ~8 µg/mL dilute IS (e.g., 12.5 µL for each) for all but the blank - IS (if prepared). Pipette a total of 200 µL of acetonitrile into the blank - IS sample and the blank + IS. Pipette the appropriate amount of each desired working stock standard solution into separate culture tubes (e.g. 10 µL for standards D, C, B, and A; 20 µL of WSA for standard 1A; 50 µL of WSA for standard 2A; 100 µL of WSA for standard 3A; 200 µL of WSA for standard 4A). Vortex briefly to mix.

Note: The blank - IS is also known as a double blank and the blank + IS is also known as a blank.

5.3 Pipette acetonitrile (e.g., 5 mL) into each tube. Cap each tube, then vortex ~2 minutes in the IKA- Vibrax® shaker at the maximum setting.

5.4 Centrifuge (e.g., 3500 rpm) for about 10 minutes. Transfer the supernatant into clean glass culture tubes. Evaporate to dryness (e.g. ~60 min in the Turbo-Vap®) with a gentle stream of nitrogen and moderate heat (e.g. 35 °C).

5.5 To the dried residue in the tube, add 200 µL of 0.2% Formic acid in methanol and 50 mL of organic-free water. Vortex vigorously, then filter (e.g., 0.2 µm PVDF) the contents of each culture tube into a conical autosampler vial. Cap the vial. If

ANALYTICAL METHOD

Method No.: BACG-3495

Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

necessary, tap the vial to dislodge any entrapped air in the bottom of the vial. Analyze under established instrumental conditions.

5.6 Summary of standard concentrations:

Standard Level	Approximate Concentration (ng/mL)
4A	2000
3A	1000
2A	500
1A	200
A	100
B	50
C	20
D	10
Blank	0

6.0 PREPARATION OF SAMPLES

6.1 Allow each plasma sample to thaw to room temperature. Vortex each sample briefly, but vigorously. Pipette an aliquot of each sample (e.g., 500 µL) into individual microcentrifuge tubes.

6.2 Pipette the IS (e.g., 12.5 µL) into each sample or QC. Vortex briefly to mix.

6.3 Pipette acetonitrile (e.g., 5 mL) into each tube. Cap each tube, then vortex ~2 minutes in the IKA- Vibrax® shaker at the maximum setting.

6.4 Centrifuge (e.g., 3500 rpm) for about 10 minutes. Transfer the supernatant into clean glass culture tubes. Evaporate to dryness (e.g. ~60 min in the Turbo-Vap®) with a gentle stream of nitrogen and moderate heat (e.g. 35 °C).

ANALYTICAL METHOD

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Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

6.5 To the dried residue in the tube, add 200 µL of 0.2% Formic acid in methanol and 50 µL of organic-free water. Vortex vigorously, then filter (e.g., 0.2 µm PVDF) the contents of each culture tube into a conical autosampler vial. Cap the vial. If necessary, tap the vial to dislodge any entrapped air in the bottom of the vial. Analyze under established instrumental conditions.

7.0 ANALYSIS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY / MASS SPECTROMETRY (HPLC/MS)

7.1 Conditions are to be optimized if necessary.

7.1.1 Pump and Autosampler Conditions

Flow rate:	400 µL/min
Injection volume:	100 µL
Column:	Aquasil C18 HPLC column (250 mm x 2 mm) Keystone Scientific (Cat. No. 255-775-2)
Guard Column:	Javelin Aquasil C18 20 mm x 3 mm, Keystone Scientific
Frit:	2 µm PEEK, Upchurch Scientific
Mobile phase:	A: 0.2% formic acid in methanol
	B: deionized water

Gradient:

Time (min)	%A	%B	
0- 3	80	20	
3 - 4	95	5	(Step gradient)
4- 6	95	5	
6 - 10	20	80	(Linear)

7.1.2 PE Sciex API 150EX Single Quadrupole Mass Spectrometer Conditions
Software: PE Sciex MassChrom and TurboQuan

ANALYTICAL METHOD

Method No.: BACG-3495

Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

Heated Nebulizer source conditions

Note: Values listed under "MS Acquisition Conditions" override parameters in this table.

Auxiliary Gas: Air at 85 psi

Parameter	Value
IS	0
NC	4.00
TEM	450
OR	20
RNG	100
Q0	-2
IQ1	-3
ST	-8
RO1	-4
DF	-300
CEM	2000
NEB	10
CUR	6
QPE	0
POL	0

ANALYTICAL METHOD

Method No.: BACG-3495

Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

Parameter	Value
VCM	0
IPE	0

MS/MS Acquisition Conditions

Scan type: SIM
Polarity: Positive
Acquisition mode: Profile
Pause time: 5 milliseconds

Masses requested:

Artelinic Acid :

Q1 Mass (amu)	Dwell Time (ms)
221.2	100
249.2	100
267.2	100
419.3	100

Artesunic Acid:

Q1 Mass (amu)	Dwell Time (ms)
221.2	100
249.2	100
267.2	100
419.3	100

ANALYTICAL METHOD

Method No.: BACG-3495

Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

8.0 CALCULATIONS

8.1 At the end of the analytical run, review each chromatogram to ensure the retention time, peak shape, and peak height and peak area determination of the test article and the IS are acceptable. Manual integration may be used as necessary to improve integration of peaks. For quantitation , use the ion profiles at the following mass-to-charge ratios:

Analyte	Ion Profile
Artelinic Acid	221.2
Artesunic Acid	221.2

8.2 Plot the peak area response of artelinic acid divided by the peak area response of the IS (artesunic acid) from all standards versus the concentration of the test article in the standards. Alternatively, the peak heights may be used instead of peak areas. Obtain the best curve fit of the data (e.g., linear fit weighted with 1/concentration of the test article or a quadratic fit). Note: The best curve fit may be dependent on the range of the standard curve and it may be necessary to have more than one standard curve for various concentration ranges. Additionally, at the discretion of the chemist, external standard quantitation may be used. This mode of quantitation should be used when unforseen matrix interferences are observed with the internal standard.

8.3 Using the standard curve, calculate the level of artelinic acid in each unknown sample. Correct the results of samples for any dilutions.

9.0 ACCEPTANCE AND REJECTION CRITERIA

9.1 Refer to SOP SRI 91-3 for acceptance/rejection criteria except acceptable accuracy for standards is 75-125% of theoretical.

ANALYTICAL METHOD

Method No.: BACG-3495

Title: Artelinic Acid in Dog Plasma: Sample Preparation and Analysis by High Performance Liquid Chromatography/ Mass Spectrometry (HPLC/MS)

10.0 REPORTING

10.1 Results of all analyses are tabulated, and the raw data, original chromatograms, and reports are to be filed in the appropriate study file.

Author: _____ 10/12/99

Greg S. Gorman Ph.D.
Research Chemist

Date

Approved by: _____ 10/12/99

James D. Johnson, Manager
Bioanalytical Chemistry Group

Date

Appendix C

Dose-Range Finding Study of Injectable Artelinate and Artesunate in Rats:

Report from Dr. Qigui Li at WRAIR
Plasma Concentrations of DQHS and Arteether

Male dogs Jan-00

Walter Reed Army Institute of Research

Department of Pharmacology

Data report for the female dogs EM2597 and EM2603.
Plasma samples from SRI (Protocol No. 7869.14.01)

EM2597			
Time	Tube No.	DQHS	AE
(h)*		(ng/ml)	(ng/ml)
D1 0	1	0	**0**
D1 0.25	2	12.90	**41.46**
D1 0.5	3	12.93	**51.99**
D1 1	4	9.02	**79.86**
D1 2	5	4.43	**80.84**
D1 8	6	16.51	**41.34**
D1 4	7	6.30	**17.49**
D7 144	8	20.65	**130.72**
D14 312	9	14.38	**158.76**
D14 312.25	10	21.15	**212.39**
D14 312.5	11	31.82	**300.02**
D14 313	12	32.30	**227.84**
D14 314	13	27.63	**194.21**
D14 316	14	17.05	**191.87**
D14 320	15	13.40	**101.27**

EM2603			
Time	Tube No.	DQHS	AE
(h)*		(ng/ml)	(ng/ml)
D1 0	1	0.00	**0.00**
D1 0.25	2	11.17	**34.82**
D1 0.5	3	5.87	**42.37**
D1 1	4	11.21	**83.36**
D1 2	5	14.05	**42.67**
D1 8	6	11.84	**34.24**
D1 4	7	6.72	**17.47**
D7 144	8	1.32	**126.29**
D14 312	9	41.39	**150.22**
D14 312.25	10	34.93	**220.92**
D14 312.5	11	22.52	**236.33**
D14 313	12	25.50	**254.66**
D14 314	13	26.83	**262.54**
D14 316	14	14.65	**160.41**
D14 320	15	47.92	**104.16**

Notices:

1) These samples were analyzed with modified Method 2 as moble phase 40/60 to 25/75, and DQHS n-Propyl Ether as an internal standard.
2) Coef. of determination, $r^2 = 0.9996$ for DQHS; Coef. of determination, r2 = 0.9972 for AE
3) Each sample in Standard curves was in 20% of its respective nominal value.

QC samples	No	DQHS	AE
		(ng/ml)	(ng/ml)
25 ng/ml	A	22.4	24.6
25 ng/ml	B	17.9	25.6
100 ng/ml	C	102.6	101.4
100 ng/ml	D	101.1	97.5
250 ng/ml	E	243.2	253.5
250 ng/ml	F	249.7	255.4

File name: A-SRI-AE
Samples received on Oct., 1999?
Samples extracted on Nov. 20, 1999
Samples acquired on Nov. 24, 1999
by SPC Jennifer Rotger
Reporting by Dr. Qigui Li, on Jan. 12, 2000

Walter Reed Army Institute of Research

Department of Pharmacology

Data report for the female dogs EF2610 and EF2615.
Plasma samples from SRI (Protocol No. 7869.14.01)

EF2610			
Time (h)*	Tube No.	DQHS (ng/ml)	AE (ng/ml)
D1 0	1	0	**0**
D1 0.25	2	6.00	**18.33**
D1 0.5	3	13.64	**50.25**
D1 1	4	9.44	**74.58**
D1 2	5	4.57	**79.74**
D1 8	6	12.58	**41.84**
D1 4	7	6.06	**18.17**
D7 144	8	21.59	**131.05**
D14 312	9	13.08	**130.77**
D14 312.25	10	18.58	**184.20**
D14 312.5	11	23.38	**231.73**
D14 313	12	35.65	**247.52**
D14 314	13	26.99	**187.72**
D14 316	14	14.53	**144.46**
D14 320	15	13.02	**110.09**

EF2615			
Time (h)*	Tube No.	DQHS (ng/ml)	AE (ng/ml)
D1 0	1	0.00	**0.00**
D1 0.25	2	9.64	**34.23**
D1 0.5	3	10.41	**49.32**
D1 1	4	11.44	**75.13**
D1 2	5	14.03	**42.67**
D1 8	6	6.23	**32.81**
D1 4	7	8.27	**18.27**
D7 144	8	11.52	**128.52**
D14 312	9	38.81	**148.33**
D14 312.25	10	25.87	**180.75**
D14 312.5	11	25.24	**271.04**
D14 313	12	17.19	**493.52**
D14 314	13	14.31	**163.67**
D14 316	14	13.10	**131.28**
D14 320	15	4.39	**76.11**

Notices:

1) These samples were analyzed with modified Method 2 as moble phase 40/60 to 25/75, and DQHS n-Propyl Ether as an internal standard.
2) Coef. of determination, $r^2 = 0.9989$ for DQHS; Coef. of determination, r2 = 0.9995 for AE
3) Each sample in Standard curves was in 20% of its respective nominal value.

QC samples	No	DQHS (ng/ml)	AE (ng/ml)
25 ng/ml	A	21.4	26.1
25 ng/ml	B	21.0	25.4
100 ng/ml	C	99.6	102.6
100 ng/ml	D	101.4	105.5
250 ng/ml	E	247.7	249.8
250 ng/ml	F	243.4	246.2

File name: A-SRI-AE
Samples received on Oct., 1999?
Samples extracted on Nov. 14, 1999
Samples acquired on Nov. 22, 1999
by SPC Jennifer Rotger
Reporting by Dr. Qigui Li, on Jan. 11, 2000